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AN INDEPENDENCE OF INTENSITY FOR EMBRYO DOMINANT LETHALS INDUCED BY X-RAYS IN FIRST MEIOTIC METAPHASE EGGS OF HABROBRACON[1]

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Received February 1, 1960

WHEN Habrobracon eggs are irradiated and subsequently fertilized, the decrease in hatchability is attributed to dominant lethal mutations. Cytoplasmic injury is not lethal at low doses of irradiation (WHITING 1955). If eggs are irradiated in first meiotic metaphase and allowed to develop parthenogenetically, they show a linear decrease in hatchability to an increase in dose (WHITING 1945a; HEIDENTHAL, CLARK and GOWEN 1955). Due to the linear relation of hatchability to dose in unfertilized eggs, it was assumed that injuries to metaphase I eggs were in the form of terminal deletions or minute interstitial deletions, both one-hit events. Furthermore, if two-hit events such as interchanges constituted a significant proportion of the dominant lethals, it would be expected that bridges would be found in the first division. The excellent cytological studies of WHITING (1945a,b) have shown that fragments, but no bridges, are present in division I. Bridges are evident in large numbers in the second meiotic division indicating the fusion of sister chromatids after chromosome breakage. Fusion of homologous chromatids would lead to bridge formation in the first division.

The absence of any intensity effect and the linearity of a dosage response curve has been explained as being caused by chromosome breakage and deletions followed by sister strand fusion and bridge formation during the first cleavage division (PONTECORVO 1942; WHITING 1945). These findings led WHITING to conclude that most dominant lethality in metaphase I eggs was caused by a single ionizing track. At the time, quantitative data on the intensity effect were not available although preliminary experiments had shown no evidence of any intensity factor when hatching of unfertilized eggs was the criterion of damage subsequent to irradiation of metaphase I eggs (WHITING 1945a).

The present experiment was designed to extend the early studies of WHITING (1945a) on the effects of intensity using the induction of dominant lethal mutations as the criterion of damage. In the present experiments a total dose of 800r was administered in intervals ranging from one minute to two hours. It was hoped that if any of the dominant lethals induced in metaphase I eggs were the result of interchromosomal exchanges, these intensity experiments would indicate the proportion of such events in relation to the one-hit events. Thus, in an intensity experiment the difference between the damage induced when the dose is ad-

[1] Research carried out at Brookhaven National Laboratory under the auspices of the U.S. Atomic Energy Commission.

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ministered in a very short time compared to a long time is a measure of the proportion of the aberrations which are of two-hit origin.

METHODS AND RESULTS

Well-fed Habrobracon females of stock No. 33 were kept without hosts for 16 hours at 30°C before irradiation. Under these conditions the females store large numbers of eggs in the uterine sacs, all in first meiotic metaphase. Irradiation was performed with a G. E. Maxitron unit at 250 kvp with 1 mm of aluminum filtration. Differences in intensity were achieved by altering the target distance and by using 30 ma for high intensities and 6 ma for low intensity irradiations. Immediately after irradiation, some of the irradiated virgin females were placed with prestung hosts for tests of the hatchability of unfertilized eggs which normally develop into haploid males. Some of the females were mass mated for a period of one half to one hour to males of the 17 o^i stock before being placed with host caterpillars. Females and host caterpillars were kept in an incubator at 30°C for six hours. Exhaustive tests have shown that under these conditions only those eggs irradiated in first metaphase are laid (WHITING 1940). After this period females were discarded and the eggs were collected, placed in mineral oil and allowed to develop for 42 hours at 30°C before hatchability was scored.

The method for estimating the number of dominant lethals induced by the radiation treatment has been previously described (ATWOOD, VON BORSTEL and WHITING 1955) and makes use of the equation:

$$D = 1 - \frac{m - V_u(1-f)}{f}$$

where m is the hatchability of eggs from fertilized females, V_u is the viability of the unfertilized eggs from unmated females and f is the proportion of eggs fertilized. This proportion was determined by means of a sex ratio experiment and found to be approximately 0.60 in this cross. D expresses the frequency of chromosome sets having *at least one* dominant lethal. The total irradiation dose was kept constant at 800r while the intensity of the irradiation was varied from 800r to 6.6r per minute.

Each experiment was repeated at least twice, and the pooled data for each intensity are presented in Table 1. These results are plotted in Figure 1 and a least squares line fitted to the experimental points. A t test applied to the data for the extremes of the range investigated showed that there is no significant difference in the number of dominant lethals induced by 800r of X-rays delivered in one minute or in 121 minutes. It is evident from the data that the induction of dominant lethals in metaphase I eggs of Habrobracon is completely independent of the intensity at which the irradiation is administered. These data are in agreement with the experiments of WHITING (1945a) on hatchability of unfertilized eggs and show that even at high dose rates dominant lethals in metaphase I eggs are strictly attributable to one-hit events. If any significant portion of the dominant lethals are due to multihit events, then this proportion must be too small to be detected by the methods presently available.

TABLE 1

Dominant lethals (D) *induced in metaphase I eggs by 800r of X-rays*

Time of irradiation (min)	Intensity r/min	Total eggs	Hatchability of eggs from: unmated ♀ V_u	Hatchability of eggs from: mated ♀ m	Dominant lethals D	S.E. of D
1	800	675	.167	.306	.602	.046
4	200	881	.267	.335	.620	.038
8	100	920	.215	.323	.605	.039
10	80	534	.223	.365	.540	.051
16	50	682	.264	.399	.512	.066
20	40	1,059	.221	.356	.553	.035
30	26.6	641	.341	.448	.490	.052
40	20	278	.259	.385	.532	.072
53.5	15	883	.302	.398	.538	.041
62	13	512	.329	.411	.535	.052
121	6.6	374	.331	.421	.518	.065
		7,412				

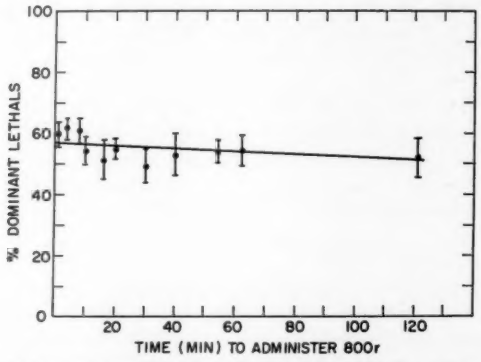

FIGURE 1.—The relation of dose-rate to embryo dominant lethal mutations induced by 800r of X-rays in metaphase I eggs of Habrobracon. A least squares line is fitted to the experimental points. The slope of the line is not significant.

It is of interest in this connection to note that the dose-injury curves for dominant lethality in the spermatozoa of Drosophila (SONNENBLICK 1940; DEMEREC and FANO 1944) Habrobracon (HEIDENTHAL 1945) and for the honeybee (LEE 1958), all show evidence of dose-rate dependence. The data indicate that two-hit events occur at high doses and one-hit events at low doses.

In the present experiments (Table 1) the hatchability of eggs from mated females was consistently higher than that from unmated females receiving the same radiation treatment, indicating that in unfertilized eggs a proportion of the unhatched eggs contain recessive lethals. These results are in agreement with the earlier findings summarized by ATWOOD, VON BORSTEL and WHITING (1956).

SUMMARY

Dominant lethals induced in metaphase I eggs of Habrobracon are chromosomal in nature and are dose-rate independent, indicating only one-hit events.

ACKNOWLEDGMENTS

The author is indebted to Dr. Phelps Crump for statistical advice; to Miss Liza McCormick for technical assistance; and to Drs. A. R. Whiting, D. S. Grosch, and Dale M. Steffensen for critically reading the manuscript and offering helpful suggestions.

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HETEROGENEOUS CLONES ("HETEROCLONES") IN STREPTOMYCES COELICOLOR A 3(2)

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Received April 6, 1960

CERTAIN recombinant colonies of *Streptomyces coelicolor* (*S. violaceoruber* according to KUTZNER and WAKSMAN 1959) display a mixture of different phenotypes (SERMONTI and SPADA-SERMONTI 1959). Experimental conditions have been successfully established for the recovery of such colonies, and a convenient method has been developed for the genetical analysis of *S. coelicolor*. To describe these colonies and their descendants, it has been necessary to adopt a new term: "heteroclone," which describes a clone stemming from a genetically heterogeneous and highly unstable original unit. This term can readily be applied to the descendants of a bacterial zygote (LEDERBERG 1957; ANDERSON 1958).

MATERIALS AND METHODS

The strains employed were kindly supplied by DR. HOPWOOD of Cambridge, and were those which he used in previous work (HOPWOOD 1959). All of them were derived from *Streptomyces coelicolor* A 3(2). According to HOPWOOD (1959) the markers adopted are linked thus:

me-2 — 4 — *his-1* — 21 — *arg-1* — ... ... — *st-1*

The numbers below the line indicate, in recombination units, the supposed distance between adjacent markers. *me*, *his* and *arg* indicate loci involved in the synthesis of methionine, histidine and arginine, respectively; the locus *st* governs resistance to streptomycin. The locus symbol will be used to indicate the mutant allele, while the sign + will indicate the wild type allele. *st* signifies resistance to streptomycin. The replica plating method was adopted for the characterization of strains. Recombinants were obtained by the routine procedure (SERMONTI and SPADA-SERMONTI 1959): the two parental strains being mixed on complete medium, then the conidia of the mixed culture being plated on selective media.

RESULTS AND DISCUSSION

We have repeated the "crosses" carried out by HOPWOOD (1959) to verify linkage between the genes examined, and the results were fundamentally consistent with those presented by HOPWOOD.

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Heteroclones have been selected among prototrophic recombinant colonies derived from those crosses in which *me* and *his* were in repulsion. The selected colonies were those which developed after plating on minimal medium but could not be reproduced by replica plating on fresh minimal medium. In general, such colonies were small compared with the genuine recombinants. The conidia of some of these colonies have been harvested and plated onto complete medium (and/or complete medium containing streptomycin), and the colonies obtained have been classified by replica plating on variously supplemented media. In this way a great variety of segregant subclones have been obtained from every heteroclone—up to a dozen of the 16 possible types (Tables 1 and 2). Among

TABLE 1

Analysis of heteroclones from crosses: me + arg st × + his + +

(A) Streptomycin-sensitive segregants

Heteroclones (code no.)*	36–5	36–7	36–8	38–1	38–6	Totals Actual	Totals Percent	Expected freq.†
Segregant phenotypes								
+ *his* +	39	39	32	37	40	187	54	37.9
me + *arg*	14	9	6	31	60	120	35	
me + +	1	3	7	3	2	16	5	10.1
+ *his arg*	5	1	0	3	9	17	5	
+ + *arg*	0	0	1	1	2	4	1	1.6
me his +	0	0	1	0	0	1	0	
+ + +‡	0	0	0	0	0	0	0	0.4
me his arg	0	0	0	0	0	0	0	

(B) Streptomycin-resistant segregants

Heteroclones (code no.)*	36–5	36–2	36–8	Totals Actual	Totals Percent	Expected freq.†
Percent of sensitives	0.04	0.05	0.6			
Segregant phenotypes						
+ *his* +	9	11	16	36	33	37.9
me + *arg*	19	15	10	44	40	
me + +	11	5	0	16	15	10.1
+ *his arg*	1	1	2	4	4	
+ + *arg*	1	0	0	1	1	1.6
me his +	3	1	0	4	4	
+ + +	1	3	1	5	5	0.4
me his arg	0	0	0	0	0	

* The first number indicates the cross, the second the clone.
† Assuming normal meiotic segregation, according to the average linkage data of Hopwood (1959), in the absence of interference.
‡ Rare prototrophic segregants ($<10^{-3}$) appearing on minimal medium: the majority were streptomycin-sensitive.

TABLE 2

Analysis of heteroclones from cross: + his arg st × me + + +

Heterclones (code no.)	Streptomycin-sensitive segregants			Streptomycin-resistant segregants		
	41–2	41–3	41–7	41–2	41–3	41–7
Total frequency among segregants	69 percent	97.5 percent	99.5 percent	31 percent	2.5 percent	0.5 percent
Segregant phenotypes						
+ *his arg*	48	34	33	22	23	47
me + +	43	31	32	12	17	0
+ *his* +	6	21	31	12	17	0
me + *arg*	2	0	0	0	0	0
+ + *arg*	0	1	0	0	0	0
me his +	0	0	0	0	0	0
+ + +	3	13	9	0	16	0
me his arg	0	0	0	0	0	0

segregants of the cross *me* + *arg st* × + *his* + +, the allele *st* was present with very low frequency (< 1%), but it was always present. The streptomycin-resistant segregants are tabulated separately (Table 1B). If the character *st*/+ is ignored, the frequency of the segregant classes approximated that expected on the basis of linkage deduced from standard analysis (HOPWOOD 1959). The two parental classes *me* + *arg st* (Table 1B) and + *his* + + (Table 1A) occurred with widely different frequencies, the lack of the first type being a reflection of the considerable deficiency of the *st* allele. The parental combinations of the three nutritional characters were present in roughly equivalent frequencies (more alike in cross 38, see Table 1A) among both sensitive and resistant segregants. The other complementary combinations of nutritional markers were also present with frequencies which were, on the whole, comparable. But if the character *st*/+ was considered, the streptomycin-resistant segregants were present in grossly deficient numbers in every case.

A situation was observed among the segregants from the heteroclones of the cross + *his arg st* × *me* + + + (Table 2) which was analogous to that described in the previous cross. Parental combinations of nutritional markers are those which occur with the highest frequency, and there is again a great deficiency of the *st* marker. Certain peculiarities are worthy of note: in one of three heteroclones analysed (41–2) the *st* marker was present with high frequency, in about one third of the segregants; there is a prevalence of the type + *his* + over the complementary type *me* + *arg* and an excess of prototrophs. These asymmetries cannot be explained at the present stage of the analysis.

Some heteroclones give rise only to parental types, with approximately equal frequency, and sometimes are accompanied by rare recombinants. These would appear to be derived from heterokaryotic units.

Rarely, prototrophic colonies could be obtained when conidia from a hetero-

clone were plated on minimal medium (order of frequency 10^{-3} to 10^{-4} in the cross *me* + *arg st* × + *his* + +). Some of these were true stable prototrophic recombinants, while others were subheteroclones which could not be transferred by replica-plating onto fresh minimal medium. These second-order heteroclones exhibited a reduced range of phenotypes and sometimes the complete absence of certain markers.

The simplest interpretation of the nature of heteroclones is that they originate from plating units containing a highly unstable heterozygous nucleus. During the multiplication of such a nucleus, frequent segregation of alleles would occur at the marked loci. A similar process, carried through to completion in the mixed culture on complete medium, may be the way by which "normal" pure recombinant clones arise.

At this stage of the work the possibility cannot be excluded that heteroclones are of secondary origin: that is, that they are produced by the interaction of conidia, or occasional hyphae, after plating onto minimal medium. Whatever the correct interpretation may be, heteroclonal colonies growing on a minimal medium certainly result from some form of co-operation between the genotypes of the parent strains, and hence provide a test for allelism in *S. coelicolor*.

SUMMARY

When plated on minimal medium, conidia from a mixed culture on complete medium of *S. coelicolor* A 3 (2) give rise to rare heterogeneous colonies or "heteroclones." On replating, these exhibit a great variety of segregant subclones. The frequency of the various segregant classes is close to that predicted by linkage data obtained by standard analysis (HOPWOOD 1959), except for streptomycin-resistant segregants which are very deficient in numbers. It is supposed that heteroclones are initiated from heterozygous nuclei which undergo rapid segregation. Heteroclones can provide a useful tool for genetical analysis, and may furnish a test of allelism in *S. coelicolor*.

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LINKAGE STUDIES IN GOSSYPIUM. II. ALTERED RECOMBINATION VALUES IN A LINKAGE GROUP OF ALLOTETRAPLOID G. HIRSUTUM L. AS A RESULT OF TRANSFERRED DIPLOID SPECIES GENES[1]

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Received November 24, 1959

ACCORDING to STEPHENS (1950) speciation in the genus Gossypium occurs both by cryptic structural differentiation of chromosomes and by major gene substitution and mutation. In the first paper of this series RHYNE (1958) reported a sizeable reduction of recombination values within linkage groups following a transfer of intact diploid Gossypium linkage groups into allotetraploid *G. hirsutum*. These results show that chromosomal structural differences are more important in the altering of genetic recombination than is the influence of genotypic background. GERSTEL (1956) and GERSTEL and PHILLIPS (1958) indicate that chromosomal structural differences were in part responsible for decreasing the segregation of marker genes in hexaploid hybrids of *G. hirsutum* × Gossypium diploid species. The segregation ratios were larger in the hexaploid hybrids $2A_hD_hD_x$, in which *G. hirsutum* L. $2A_hD_h$ and wild American D diploids species were involved, than the expected ratio of five dominant to one recessive for duplex genes in the autotetraploid D genome chromosomes. Each of these studies thus conforms to one of the criteria which STEPHENS (1950) proposed for indicating cryptic structural differentiation among chromosomes in Gossypium interspecific hybrids.

The assumption is, therefore, that structural differences exist among chromosomes of diploid and allotetraploid Gossypium species, and that diploid structural arrangements can be incorporated into *G. hirsutum* chromosomes by an insertion of genes and segments of diploid linkage groups into *G. hirsutum* linkage groups. Possibly these inserted diploid structural arrangements could alter recombination values within the *G. hirsutum* linkage groups. One expected alteration should be a reduction of recombination values in certain regions of a linkage group. Such a reduction was reported by RHYNE (1958) for intact, diploid, linkage groups transferred into *G. hirsutum*. A second expected alteration could be an increase of recombination values in regions of a linkage group adjacent to structural arrangements. Such an increase of recombination values in regions adjacent to inversions in maize chromosomes was assembled by SWANSON (1957). The present paper reports on the insertion of genes and associated structural arrangements of diploid

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Gossypium species into a linkage group of *G. hirsutum* and records the concomitant alteration of recombination values within the linkage group. A possible explanation for the observed alteration of recombination values is offered.

METHODS AND MATERIALS

In order to measure the alteration of recombination values caused by the insertion of diploid genes and structural arrangements, a linkage group must have three or more known loci. Three linkage groups in *G. hirsutum* are known to have three or more linked loci, but only one is useful for this study. It carries the loci *Cl1–R1–Yg1–dw*; but *dw* is of limited use because of classification difficulties since no known allele occurs within *G. hirsutum*. The linkage group is in the D_h genome of allotetraploid *G. hirsutum* $2A_hD_h$. Genes and segments of linkage groups were obtained from the wild American diploid *G. raimondii* Ulbr., $2D_5$, and *G. armourianum* Kearney, $2D_2$, by using hexaploid hybrids $2A_hD_hD_5$ and trispecies hybrids $A_hA_2D_hD_5$ or D_2. Allelic genes from the diploids were then substituted at the various loci of the D_h linkage group using the backcross technique with *G. hirsutum* as the recurrent ovule parent. In the study the genes used are given as follows, omitting the subscript "one":

cl—cluster fruiting habit, a *G. hirsutum* recessive allele.

Cl—normal fruiting habit, the *G. hirsutum* dominant allele.

Cl^{rai}—normal fruiting habit, from *G. raimondii* $2D_5$, and dominant to *cl*.

r—"green" plant parts, clear petal margin, no anther or filament coloration; a *G. hirsutum* recessive allele.

R—red plant parts, red petal margin, no anther or filament coloration; an incompletely dominant *G. hirsutum* allele of *r*.

r^{rai}—green plant parts, clear petal margin, red anthers and filaments, red petal spot. The allele is dominant to *r*. It was derived from *G. raimondii* $2D_5$. An R/r^{rai} phenotype is red plant parts, red anthers and filaments, red petal margin and petal spot. This phenotype was most useful in the synthesis of experimental stocks and in the classification of plants of F_2 populations, as is illustrated in the experiment.

r^{arm}—when in an otherwise hirsutum background, can be distinguished from *r* by the presence of red anther cases. When transferred in the intact *Cl-R-Yg* linkage group from *G. armourianum* $2D_2$, the allele imparts red anther and filament coloration and a large red petal spot. It interacts with *R* in a fashion similar to r^{rai}.

yg—yellow-green cotyledons and true leaves in the presence of *yg2*, a *G. hirsutum* mutant gene.

Yg—green cotyledons and true leaves in the presence of *yg2;* a *G. hirsutum* dominant gene.

Yg^{rai}—green cotyledons and true leaves in the presence of *yg2;* a gene dominant to *yg* and obtained from *G. raimondii*.

dw—white lint and seed fuzz; a *G. hirsutum* gene having no known allele within the species.

dw^{rai}—dirty-white lint and tan seed fuzz; a gene incompletely dominant to *dw* and derived from *G. raimondii*.

The genes *yg1* and *yg2* are found in the D and A genomes of *G. hirsutum*, respectively. Each gene must be in homozygous condition to produce a yellow-green phenotype. The dominant *Yg1* is less common than *Yg2*. Many stocks including some used in these experiments have the *Yg2Yg2 yg1yg1* genotype which is indistinguishable in phenotype from the *yg2yg2 Yg1Yg1* genotype used herein. For presentation purposes the loci of the A_h genomes are omitted unless they are needed in genotypes to describe particular phenotypes.

The D_h chromosome was marked generally with the linkage *cl-R-yg-dw*, the D_5 by *Cl*^{rai}-*r*^{rai}-*Yg*^{rai}-*dw*^{rai}, and the D_2 by *Cl*^{arm}-*r*^{arm}-*Yg*^{arm}-*dw*^{arm}. The diploid linkages were transferred intact by three to 12 backcrosses into the *G. hirsutum* standard stock. The D_5 diploid genes and associated structural arrangements were then inserted into the *G. hirsutum* linkage group and transferred intact to the 12th backcross generation. This procedure reduced the number of diploid genes in other D_h chromosomes. (Subsequently the series of backcrosses proved to be unessential, for D_5 and other diploid genes in other chromosomes had little if any effect on the recombination values of the linkage group undertest. The results of the first backcross were comparable to those of the later backcrosses.) The reported data from the 14th and later backcrosses are representative of the alteration of recombination values within a *G. hirsutum* linkage, caused by genes and structural arrangements of other Gossypium species when inserted into the linkage group.

The following genotypes were provided by appropriate genetic procedure:

Genotype A, $\frac{Cl\text{-}r\text{-}Yg\text{-}dw}{cl\text{-}R\text{-}yg\text{-}dw}$, was a control D_h chromosome in the genotypic background of genotypes B and C below. Recombination was measured at *Cl-r* and *r-Yg* by backcrossing.

Genotypes B and C were most readily obtained by growing a large F_2 from the 12th backcross hybrid $\frac{cl\text{-}R\text{-}yg\text{-}dw}{Cl^{rai}\text{-}r^{rai}\text{-}Yg^{rai}\text{-}dw^{rai}}$ and inserting genes and segments into the D_h linkage by crossing over. Recombination in each of the regions of the D_h linkage is much reduced and double recombination has not been detected. Plants of the genotype $\frac{cl\text{-}R\text{-}Yg^{rai}\text{-}dw^{rai}}{Cl^{rai}\text{-}R\text{-}yg\text{-}dw}$ having a crossover between *R* and *Yg*^{rai} in one gamete and between *Cl*^{rai} and *R* in the other gamete were desired. These plants then were crossed to appropriate lines of the standard *G. hirsutum* stock to obtain

Genotype B, $\frac{cl\text{-}R\text{-}Yg^{rai}\text{-}dw^{rai}}{Cl\text{-}r\text{-}yg \quad\quad \text{-}dw}$, which provided the *Yg-dw* segment of D_5 in a *G. hirsutum* linkage group. Recombination was measured at *Cl-R* and *R-yg* in F_2 populations by the method of Murty (1954). The *yg-dw* linkage was of little use since its recombination value within *G. hirsutum* is not known and *yg-dw*^{rai} recombination fluctuates depending on the "genes" at the adjacent *cl-R-yg* loci.

Genotype C, $\frac{cl\text{-}R\text{-}Yg^{rai}\text{-}dw}{Cl\text{-}r\text{-}yg \quad \text{-}dw}$, provided the *Yg* segment of D_5 in a *G. hirsutum* linkage group. Recombination was measured at *Cl-R* and *R-yg* as in genotype B.

Genotype D, $\frac{Cl^{rai}\text{-}R\text{-}Yg^{rai}\text{-}dw^{rai}}{cl \quad \text{-}r\text{-}yg \quad \text{-}dw}$, provided the *R* segment of the D_h linkage in a D_5 chromosome. Recombination was measured at *Cl-R* and *R-yg* as in genotype B.

The remaining genotypes were obtained in comparable genetic backgrounds. The *Yg1* allele of the D_h linkage was absent from many genotypes since the appropriate testers having *Yg1* were unavailable at the beginning of the synthesis:

Genotype E_1, $\frac{Cl\text{-}r^{arm}\text{-}Yg\text{-}dw}{cl\text{-}R \quad \text{-}yg\ \text{-}dw}\ \frac{Yg2}{yg2}$, provided the r^{arm} segment of the D_2 linkage in a D_h chromosome. It was paired with

Genotype E_2, $\frac{Cl\text{-}r\text{-}yg\text{-}dw}{cl\text{-}R\text{-}yg\text{-}dw}\ \frac{Yg2}{yg2}$, which provided a D_h linkage in the E genotypic background.

Genotype G_1, $\frac{cl\ \text{-}r^{rai}\text{-}yg\text{-}dw}{Cl\text{-}R \quad \text{-}yg\text{-}dw}\ \frac{Yg2}{yg2}$, provided the r^{rai} segment of D_5 in a D_h chromosome. It was paired with

Genotype G_2, $\frac{cl\ \text{-}r\ \text{-}yg\text{-}dw}{Cl\text{-}R\text{-}yg\text{-}dw}\ \frac{Yg2}{yg2}$ which provided a D_h linkage in a G genotypic background.

Genotype H, $\frac{Cl\text{-}r\text{-}Yg\text{-}dw}{cl\text{-}R\text{-}yg\text{-}dw}$, provided an independent estimate of the control D_h linkage in the year that genotypes D and E were grown. The D_h chromosome *Cl-r-Yg-dw* was transferred to the common A genotypic background from Hopi Moencopi, a *G. hirsutum* cotton but not of Upland ancestry. Its *r* allele is actually recessive to the *r* Upland allele in genotypes A, E_2 and G_2.

The hybrids of the various genotypes were grown in the winter at Iguala, Mexico, where backcrossing facilities are limited. The use of backcrossing was restricted to the control genotypes A and H in order to increase population size. Coupling phase F_2 linkage was highly efficient for estimating recombination in regions 1(*Cl-R*) and 2(*R-Yg*) and large populations were obtained by self-pollinating the hybrid plants. Classification was done in the summer at Clayton, North Carolina. The ease of recognizing three phenotypes for the three genotypes *RR*, *Rr*, and *rr* permitted gene *R* to be classed as either dominant or recessive to *r*. This incomplete dominance of *R* permitted the use of coupling phase for a more accurate estimation of recombination values. Even so the values for *cl-Yg* had to be obtained from repulsion phase in certain genotypes. The *cl-Yg* recombination was obtained by a calculated and an observed method since poor estimation of recombination in repulsion phase and the occurrence of double recombination reduced the accuracy of the observed value. The calculated method estimates were obtained by adding the values of regions 1 and 2, ignoring doubles. The actual frequency of doubles must be higher in the genotypes B, C, D, and E_1 than the reported observed value. The rate of doubles in F_2 was impossible to estimate accurately since all doubles were not detected, even by progeny testing.

TABLE 1

Recombination in the D_h genome linkage group (cl-R-yg-dw) *of* G. hirsutum *before and after substitution of a D_s genome "gene" at the* Yg *locus*

	Genotype tested	Source and population size	Recombination value: Region 1 (*Cl-R*)	Region 2 (*R-Yg*)	*Cl-Yg* Observed	*Cl-Yg* Calculated (region 1+2)	Observed double recombinants: Percent	Number
A	$\frac{(Cl\text{-}r\text{-}Yg\text{-}dw)}{cl\text{-}R\text{-}yg\text{-}dw}$	Backcross 1841	15.0	13.5	28.5	28.5	0.59	11
B	$\frac{(cl\text{-}R\text{-}Yg^{rai}\text{-}dw^{rai})}{Cl\text{-}r\text{-}yg \quad -dw}$	F_2 427*	28.6	10.2	27.4	38.8	0.67	1†
C	$\frac{(cl\text{-}R\text{-}Yg^{rai}\text{-}dw)}{Cl\text{-}r\text{-}yg \quad -dw}$	F_2 373*	23.0	7.3	29.6	30.3	0.00	0†

* The phenotypes associated with the genotypes *rr*, *Rr*, and *RR* are readily separable. The data were calculated as coupling phase assuming *r* dominant to *R* in region 1 and *R* dominant to *r* in region 2.

† The F_2 populations did not permit a ready detection of double recombinants although the incompletely dominant *R* gene helped in choosing suspected plants for progeny testing. The observed number in genotypes C and B was shown to be a minimum estimate since a number of suspected plants proved to have originated from double crossover gametes.

RESULTS

Effect of substitution of Yg^{rai} *for* yg: The backcross population from genotype A described in Table 1 had a recombination of 15 percent in region 1 and 13.5 percent in region 2. The expected number of doubles should be 15.0 × 13.5 percent if two chiasmata were to occur randomly between *cl* and *Yg* loci. The observed estimate was 0.59 percent for 11 doubles, five of one type and six of the other. Roughly only one third of the expected doubles were obtained. It appeared thus that one chiasma is the most common occurrence in the *cl-R-yg* portion of the chromosome. No estimate of *dw* recombination was possible.

The substitution of Yg^{rai}-dw^{rai} in genotype B resulted in 28.6 percent recombination, and the substitution of Yg^{rai} in genotype C resulted in 23.0 percent in region 1. These values are not only significantly greater than those found in the control A genotype but are greater than any intra-*hirsutum* value reported in the literature.

The recombination in region 2 for genotype B was intermediate in value between that of genotype A and genotype C. In this experiment the small population size for genotype B permits a good fit to the expectation of either the 13.5 percent of genotype A or 7.3 percent of genotype C. If 10.2 percent for genotype B occurred in a population of 1841 plants as genotype A has, this estimate would be significantly smaller than the observed 13.5 percent for value A. On the other hand, since double recombination is low and since by procedure genotypes B and C should have had the same break between *R* and Yg^{rai}, the populations of genotypes B and C can be pooled. The 8.9 percent estimate is significantly less than the 13.5 percent value of the control genotype.

The observed recombination value for *Cl-yg* was similar in each of the three genotypes; but the calculated value suggests that recombination increased significantly in genotype B and remained the same in the other two genotypes. The rate of doubles must have been higher than in genotype A despite the fact that

double recombinants are not readily detected in F_2 populations. Several of the suspected plants in the F_2 of genotype B prove by appropriate tests to be from double recombination gametes; thus, the rate of double rceombination should be considered to be higher than the reported 0.67 percent value.

Intercalated substitutions of r *alleles at the* R *locus:* The primary interest of Table 2 lies in region 1 of the genotypes since the absence of *Yg1* in genotypes E and G limited the information on recombination in region 2. The recombination values of the three control genotypes (E_2, G_2, and H) were essentially 17.0 percent, whereas the values of three substitution genotypes ranged from 26.2 to 33.5 percent. The value for each of the three substitution genotypes was significantly higher than either that of any of the control genotypes or any value given in the literature for recombination in the *cl-R* region.

At region 2 recombination was estimated for three genotypes only. The H control genotype showed a higher value for this region than usually was obtained for control genotype A in previous years. However, the value of 19.1 percent is not different from a value of 20.0 percent obtained in other *G. hirsutum* genotypes by RHYNE (1957, 1958). Control genotypes A and its hirsutum derivatives ranked consistently lower in recombination at region 2 than the H and other series did under similar conditions. The recombination values for genotypes D and E_1 were similar to that value for genotype B found in Table 1.

TABLE 2

Recombination in the D genome linkage (cl-R-yg-dw) *of* G. hirsutum *before and after substitution of various interspecific alleles at the* R *locus*

Genotype tested	Source and population size	Recombination value: Region 1 (*Cl-R*)	Region 2 (*R-Yg*)	For *Cl-Yg*: Observed	For *Cl-Yg*: Calculated (region 1 + 2)	Double recombination percent detected
D $\frac{(Cl^{rai}\text{-}R\text{-}Yg^{rai}\text{-}Dw)}{cl \quad \text{-}r\text{-}yg \quad dw}$	Backcross 71	29.6	11.3	40.9	40.9	1.40
	F_2 238	33.5	12.4	40.7	45.9	.78
E_1 $\frac{(Cl\text{-}r^{arm}\text{-}Yg\text{-}dw)}{cl\text{-}R \quad \text{-}yg\text{-}dw}$	F_2 373	31.3*	11.2†	43.3	42.5	.54†
E_2 $\frac{(Cl\text{-}r \quad \text{-}yg\text{-}dw)}{cl\text{-}R \quad \text{-}yg\text{-}dw}$	F_2 341	17.5*	...	...	...	...
G_1 $\frac{(cl\text{-}r^{rai}\text{-}yg\text{-}dw)}{Cl\text{-}R \quad \text{-}yg\text{-}dw}$	F_2 1,119	26.2*	...	...	...	...
G_2 $\frac{(cl\text{-}r \quad \text{-}yg\text{-}dw)}{Cl\text{-}R \quad \text{-}yg\text{-}dw}$	F_2 834	17.5*	...	...	...	...
H $\frac{(Cl\text{-}r \quad \text{-}Yg\text{-}dw)}{cl\text{-}R \quad \text{-}yg\text{-}dw}$	Backcross 141	17.0*	19.1	36.1	36.1	2.80

* Since *rr*, *rR*, and *RR* have separable phenotypes coupling phase linkage was used assuming *r* to be dominant to *R* in regions 1 and 2.

† Yg^a was present in this genotype requiring duplicate factor estimation of linkage and permitting only one double recombinant to be detected. The rate of doubles could be higher than the value given.

The recombination between the *Cl-Yg* loci for genotypes D and E_1 was not significantly different from the value of the control H genotype. The values 41–43 percent from D and E_1 however were greater than ordinarily expected from any D_h linkage group; a 28–30 percent value was reported for genotypes A and C of Table 1. A trend has been observed for an increase in recombination for the *Cl-Yg* loci for substitution genotypes like those of Tables 1 and 2. Population size, however, has been insufficient to provide statistical proof for an increase. Also, the necessity to measure recombination of F_2 populations in repulsion phase (genotypes B and C) and in duplicate factor segregation where *Yg2* and *Yg1* are present (genotype E_1) made linkage estimation inefficient for *Cl-Yg* loci.

DISCUSSION

A consistent reduction in the amount of recombination was reported by RHYNE (1958) in hybrids having an intact linkage group from cotton species of the A and D diploid genomes, and especially *G. raimondii* $2D_5$, inserted in amphidiploid *G. hirsutum*. In the present experiments by appropriate technique, making use of certain recombinants, the intact donor's chromosomes of the D_5 and D_2 diploid genomes were broken and portions were introduced into a *G. hirsutum* linkage group. These inserted chromosome segments contained genes which showed allelism with genes at specific loci of the *Cl-R-yg-dw* linkage group. A substitution of the D_5 segment with genes Yg^{rai}-dw^{rai} for D_h genes *Yg-dw* (genotype B, Table 1) increased recombination at the distal *cl-R* region. A "smaller" substitution of the D_5 segment with gene Yg^{rai} (genotype C, Table 1) increased recombination at distal *cl-R*, but the amount of increase was significantly smaller than the substitution at *yg-dw*. Substitution of a small D_5 or D_2 segment with its allele at the *R locus* (genotypes G_1 and E_1, respectively, in Table 2) increased recombination in the adjacent *cl-R* region. Similarly, the substitution of the *R* allele of the D_h linkage for r^{rai} in a D_5 linkage (genotype D) increased recombination in the adjacent *cl-r* region. On the other hand, a definite trend exists for a reduction in recombination in the *R-yg* region adjacent to the *cl-R* region, but only genotype B showed a significant decrease in the *R-yg* region. The *yg-dw* recombination was not reported, yet the recombination value fluctuated from genotype to genotype whenever dw^{rai} could be classified. This fluctuation would be expected if substitution in the *R-yg* region altered recombination in the adjacent *yg-dw* region as obviously it did in the *cl-R* region.

The calculated recombination values for the over-all *Cl-Yg* segment, the largest segment measureable, showed a higher estimate of recombination for substitution genotypes than for the *G. hirsutum* controls. An over-all estimate of recombination in the *cl-R-yg-dw* linkage group of *G. hirsutum* is unobtainable in the absence of known loci distal to *cl* and proximal to *dw*. The best evidence, however, suggests that this linkage group is in one arm of a D_h chromosome and that its typical chiasma number is one per arm. Two chiasmata can occur since double recombinants were observed in genotype A. But these doubles were obtained much less frequently than expected. A reduction in recombination at *R-yg* and an increase between *cl-R* would be expected if a single chiasma had shifted its position be-

cause of some unexplained chromosomal difficulty. A slight increase in the *Cl-Yg* recombination would also be expected if a single chiasma occurred within the limits of *Cl-Yg* much of the time; but the total chiasmata frequency in the *cl-R-yg-dw* arm might, but would not necessarily have to, remain constant.

Alterations of recombination which occur in regions adjacent to, or near, a structurally altered segment, such as an inversion, are discussed by SWANSON (1957, pages 257–260). An increase of recombination occurred in regions adjacent to inversions in maize where such structurally altered segments could be observed cytologically. Cytological observation of small, structurally altered chromosome segments, even if they were present, is unlikely for Gossypium chromosomes, since pachytene preparations have not been favorable for analysis.

STEPHENS (1950) outlined three criteria that would indicate structural differentiation of chromosomes in Gossypium interspecific hybrids. It also follows that the same criteria ought to hold in material obtained as a result of interspecific transference to *G. hirsutum* $2A_hD_h$. One of the more sensitive tests proposed by STEPHENS was an alteration of linkage relationships between marker genes. RHYNE (1958) showed that a reduction in linkage values was maintained in the intact linkage groups of diploid species, even after as many as 6–10 backcross generations of transference to *G. hirsutum*. In the present experiments the intact diploid linkages were broken into smaller segments by crossing over; and the small segments with their diploid alleles were associated with altered recombination within the *cl-R-yg-dw* group, even with increased recombination in the distal region having pure D_h chromosome segments. The reductions and increases of recombination in *Gossypium hirsutum* are consistent with the altered recombination, as SWANSON (1957) described, when known structural alterations occur in chromosomes of various species.

At least two instances of increased recombination may be found in the Gossypium literature on linkage relationships of interspecific gene transfers:

KNIGHT (1944) reported 32 percent recombination in the B2-B3 linkage for the first four backcrosses of *G. hirsutum* to amphidiploid *G. barbadense* L. In the fifth backcross a 48 percent value, followed by values above 40 percent in the sixth and seventh backcross generations, was obtained. STEPHENS (1950) interpreted the low value in the early backcross generations to be a reduction caused by a large segment of *G. hirsutum* chromosome that differs structurally from the chromosome of the *G. barbadense* recurrent parent. Crossing over in the fifth backcross replaced much of the *G. hirsutum* segment with homologous *G. barbadense* chromatin and consequently recombination increased in the later backcross generations.

HUTCHINSON (1946) obtained ten percent recombination in the crinkled dwarf-green lint linkage in later generations, but five percent was obtained in the early generations, of the transfer of the *G. barbadense* gene to *G. hirsutum*. In the early generations crinkle and green lint were in repulsion phase, and in the later generations a crossover placed crinkle and green lint in coupling phase.

In the two above recorded instances a portion of a linkage group from one species was intercalated into the linkage group of another species. An increased

recombination rate was observed. The procedure corresponds with the method used in the present experiments, where a portion of a diploid linkage group was intercalated into a *G. hirsutum* linkage group. The end result, an increase in recombination, therefore may be regarded as a common phenomenon, i.e., a portion of a linkage group of a donor Gossypium species alters recombination values when present in the linkage group of another species. The mechanism for altering the recombination values may be a compensatory shift in chiasma position, which results because of the presence of a structurally altered segment in a chromosome.

SUMMARY

The assumption was made that small structural differences exist among chromosomes of Gossypium species. Structural arrangements and genes of diploid Gossypium species inserted into a *G. hirsutum* linkage group should be expected to alter recombination values within the linkage group. The alteration in recombination could be both a reduction in the region containing the diploid gene and structural arrangement and an increase in the pure *G. hirsutum* regions distal to the inserted diploid gene. The substitution of diploid genes, particularly from *G. raimondii* $2D_5$ to the D_h linkage of *G. hirsutum*, specifically at the *R*, or *Yg* and *dw* loci of the *cl-R-Yg-dw* linkage group, caused genetic recombination between the *cl-R* loci to increase significantly, the *R-Yg* recombination to decrease, and the total *cl-Yg* recombination to remain the same or to show an increase as compared with the D_h control hybrids. The altered recombination, an increase in one region and a decrease in another region, corresponds to the expectation if chiasmata position were being shifted in the presence of small structurally altered segments of chromatin. A question of changed chiasma frequency was not resolved because of the limitation of available genes and loci in the D_h linkage group.

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THE GENETICS OF PAPILIO DARDANUS, BROWN. III. RACE ANTINORII FROM ABYSSINIA AND RACE MERIONES FROM MADAGASCAR

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THE present paper describes our breeding results in the two races of *dardanus* which are characterized by the presence of male-like, tailed, non-mimetic females. These races inhabit Abyssinia and Madagascar and are known as *antinorii* and *meriones*, respectively. The former is almost, and the latter is completely isolated from other races of the butterfly, and this is in contradistinction to races *cenea*, *dardanus*, *polytrophus*, *meseres* and *tibullus*, (CLARKE and SHEPPARD 1959b, 1960a) where there is considerable gene flow between adjacent populations. In this paper we refer collectively to these less isolated races as the "main African stock".

THE ABYSSINIAN RACE ANTINORII

In Abyssinia by far the commonest female form is black and pale yellow, non-mimetic, and looks much like the male (Plate No. 1). It accounts for 80 percent, and probably more, of the female population, and has tails, like all the other Abyssinian females. This is in contrast to the races of the "main African stock" where the females are always tailless. The next commonest form in race *antinorii* is *niavioides* (Plate No. 4), which is a modified *hippocoonides*, mimicking the Abyssinian form of *Amauris niavius*, but less well than *hippocoon* or *hippocoonides* mimic their respective local subspecies of the model. In addition there occurs in this race a much rarer butterfly, *f. ruspinae*, which resembles a tailed *trophonius* and mimics *D. chrysippus*. There is also a tailed *cenea* though this has only been reported twice, once in the south and once in the north of the region. It is a good mimic of *Amauris echeria*. We have also received from the Djem Djem forest a single female (unfortunately moribund) which resembled a tailed *natalica*, a form hitherto unreported from Abyssinia.

The autosomal inheritance of the female nonmimetic form: The black and yellow female form of race *antinorii* (henceforth called yellow) cannot be sex-linked on the Y chromosome because there is abundant evidence that the males carry the gene controlling this form. For example, the mother of brood 2706 (Table 1) was a female *cenea* 2530 of "main African stock", which could not have carried the yellow gene. She was mated to a male *antinorii*, and nine of her female offspring were yellow.

Furthermore, yellow cannot be sex-linked on the X chromosome because in brood 3024 (Table 1) a female heterozygous for yellow and *hippocoonides*, mated

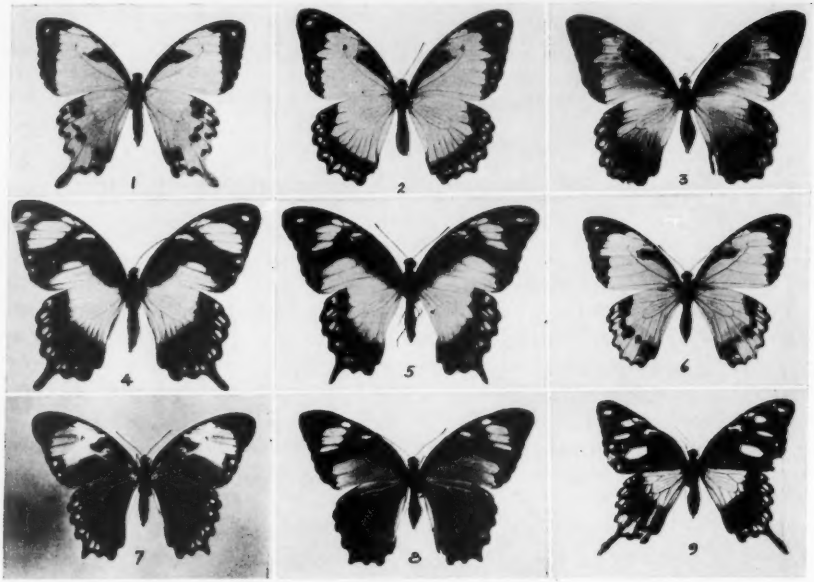

FIGURE 1.—Nonmimetic black and yellow female from Abyssinia.

FIGURE 2.—F_1 hybrid heterozygote between nonmimetic Abyssinian female (No. 1) and a *hippocoonides* from East Africa. Note the almost complete absence of a tail and the complete black band on the hindwing. The color of the pale area is yellow.

FIGURE 3.—A similar F_1 hybrid to No. 2 but heterozygous for *cenea*. Note the slightly broader black border on the hindwings and the flush of darker ochre pigment on the pale yellow areas.

FIGURE 4.—*Niavioides*, the black and white mimetic form from Abyssinia which is the tailed geographical representative of *hippocoonides* from South and East Africa and *hippocoon* from Central and West Africa.

FIGURE 5.—F_1 hybrid between the nonmimetic Madagascan race and *hippocoonides* from South Africa. The pale ground color is yellow. Note the length of the tails and the presence of a black bar dividing the pale area of the forewing into two main parts. Contrast this with No. 2 which is genetically similar with respect to the major genes controlling tail length and color pattern.

FIGURE 6.—A yellow heterozygote for either *hippocoonides* or *cenea* and the yellow Abyssinian form, obtained by backcrossing an F_1 hybrid to the Abyssinian race (Brood 2890). Contrast this insect with Nos. 2 and 3 in which the *hippocoonides* and *cenea* heterozygotes can be distinguished. The increase in the dominance of the nonmimetic pattern on backcrossing is very striking. Some sisters of No. 6 had tails and were indistinguishable from No. 1.

FIGURE 7.—An F_1 hybrid heterozygote between *trophonius* from South Africa and the yellow nonmimetic Abyssinian form. The palest parts of the pale areas are yellow and the rest of them orange brown.

FIGURE 8.—An F_1 hybrid similar to No. 7 but employing the Madagascan nonmimetic race as one of the parents (not the Abyssinian one). The pale areas are orange brown including the apical area which is somewhat paler and more orange. Note the black bar on the forewing which is absent in No. 7. A similar difference is found between No. 2 and No. 5.

FIGURE 9.—A sister of No. 6 but heterozygous for *hippocoonides* and *cenea* and homozygous for the gene for the presence of tails. An insect like this one but with much shorter tails has been caught in the Abyssinian race.

TABLE 1

Autosomal inheritance of yellow

Brood no.	Form of mother and genotype where known	Origin of father and genotype where known	Offspring				
			Males	Yellow/ *hippocoonides* females	Yellow/ *cenea* females	*cenea* females	*hippocoonides* females
2706	*cenea* main African stock H^ch	*antinorii* wild	18	5	4	3	2
				yellow			
3024	Yellow/ *hippocoonides* H^yh	main African stock	13	10	0	0	14
				yellow			

H^ch = *cenea/hippocoonides*.
H^yh = yellow/*hippocoonides*.

to a male of "main African stock" which cannot have carried the yellow, produced ten yellow females among her offspring. It is clear, therefore, that the gene controlling yellow is situated on an autosome, as are the genes controlling all the other females forms which we have investigated.

Dominance relationships and race crosses with "main African stock"

The data show conclusively that yellow is dominant to *niavioides*. Thus, in brood 2698 (Table 2) a yellow female produced 16 yellow females like herself and 11 males. Similarly, in brood 2703, a yellow female produced 13 daughters like herself and four males. However, in brood 2905, a yellow female 2703, mated to a male 2698, produced a female *niavioides*. But the evidence rests on much firmer ground than this. From its appearance it seems highly probable that *niavioides* is merely a modified *hippocoonides* (see CLARKE and SHEPPARD 1960a), and henceforth, it will often be referred to by this name. Therefore, the three broods 2707, 2711 and 2728 (Table 2) are relevant. In all of these a wild male *antinorii* was mated to a *hippocoonides* female of "main African stock" which, from its origin, could not be carrying yellow. In these three families there was segregation (not significantly differing from equality) between the yellow/*hippocoonides* (Plate No. 2) and the *hippocoonides/niavioides* hybrids. Clearly the male *antinorii* were heterozygous for *hippocoonides* (*niavioides*) which must be recessive to yellow (see Table 2).

As might be anticipated because of its rarity, we have not obtained any *cenea* from our wild *antinorii* material, but a colored photograph (sent by MR. P. R. A. MANSFIELD of the British Embassy in Addis Ababa) of a specimen caught near Filfil in Eritrea shows it to be identical with *cenea* from the "main African stock", except that it possesses short tails. It seems therefore, highly probable that the gene which controls the *cenea* pattern in the "main African stock" is the same as that which occurs in Abyssinia. In brood 2706 (Table 3) a *cenea*, known to be heterozygous for *hippocoonides* and which could not have been carrying yellow (i.e., from the "main African stock"), was mated to a wild *antinorii* male. The offspring were 18 males and four forms of female—(i) yellow/*hippocoonides*,

TABLE 2

Dominance relationship of yellow to niavioides *and* hippocoonides

Brood no.	Form of mother and genotype where known	Origin of father and genotype where known	Offspring			
			Males	Yellow/ *hippocoonides* females	Yellow females	*hippocoonides* (*niavioides*) females
2698	wild *antinorii*/ yellow	wild *antinorii*	11	0	16	0
2703	wild *antinorii*/ yellow	wild *antinorii*	4	0	13	0
2707	*hippocoonides* main African stock	wild *antinorii*	20	12	0	12
2711	*hippocoonides* main African stock	wild *antinorii*	24	15	0	7
2728	*hippocoonides* main African stock	wild *antinorii*	6	5	0	2
2905	*antinorii*/ yellow 2703	*antinorii* 2698	1	0	0	1

(ii) *hippocoonides* (i.e., *hippocoonides/niavioides*), (iii) the presumed yellow/ *cenea* (insects resembling yellow/*hippocoonides* but more ochreous, Plate No. 3) and (iv) insects which were indistinguishable in pattern from the "main African stock" *cenea*. A male from this brood (2706) mated to a South African *cenea* (see brood 2836, Table 3) gave three of the ochreous insects mentioned above, seven *cenea* and two *hippocoonides* females. In brood 3050 (Table 3) it was shown that one ochreous insect of brood 2836 was in fact yellow/*cenea*, since when it was mated with a male homozygous for *hippocoonides* (*hh*) of brood 2838, 13 *cenea* and eight yellow/*hippocoonides* were produced. Yellow, therefore, is not a recessive but in the F_1 almost a full dominant to both *cenea* and *hippocoonides*. The *hippocoonides* heterozygote with yellow has more of a complete black border to the hind wing as compared with pure yellow *antinorii* (compare Plate No. 1 and No. 2). The *cenea*/yellow heterozygote is a very similar insect but frequently more ochreous in color (Plate No. 3). From these results it can be seen that brood 2706 was a mating between a *cenea* heterozygous for *hippocoonides*, and a male heterozygous for yellow and *hippocoonides* (*niavioides*). That yellow is dominant in the *antinorii* gene complex as distinct from the F_1 hybrid comes from a consideration of brood 2890 where an F_1 female *cenea*/ *niavioides* was backcrossed to an *antinorii* male. Here the pattern and color of the yellow butterflies were completely dominant to *hippocoonides* and *cenea*, the

wing pattern being identical with *antinorii* yellow females (Plate No. 6). (See Table 3).

We have not so far been able to breed the *trophonius*-like mimic, *f. ruspinae*, but when *trophonius* from the "main African stock" was crossed with a wild *antinorii* male, two different forms of almost tailless orange butterfly resulted (brood 2741, Table 4). Some of them were identical with *trophonius* of the "main African stock", and others were bizarre insects with a large yellow patch replacing the black bar on the forewing and varying amounts of orange, brown and yellow in place of the white subapical spot (Plate No. 7). Although formal proof is lacking, it seems certain that the two forms of orange female represent *trophonius/hippocoonides* and *trophonius*/yellow hybrids respectively. If this be correct then *trophonius* of the "main African stock" is dominant to *hippocoonides* (*niavioides*) and semidominant to yellow. This is in contrast to the situation with *hippocoonides* and *cenea*, to both of which yellow is dominant. Since the bizarre insects noted above had never been found in the wild, it seemed probable that *f. ruspinae* is controlled by a different gene from *f. trophonius*. However, such a form was sent from Abyssinia in 1960 thus suggesting that *f. ruspinae* is, in fact, a modified *trophonius*.

It seems not unlikely that the nonmimetic form *natalica* does in fact occur in Abyssinia (see above). Brood 2725 (Table 4) shows the result of a mating between a *natalica* female (heterozygous for *hippocoonides*) from the "main African stock", and a yellow/*hippocoonides* (*niavioides*). Four of the eight

TABLE 3

Dominance relationship of yellow to f. cenea

Brood no.	Form of mother and genotype where known	Origin of father and genotype where known	Offspring				
			Males	Yellow/ *hippocoonides* females	Yellow/ *cenea* females	*hippocoonides* females	*cenea* females
2706	*cenea* main African stock H^ch	wild *antinorii* H^yh	18	9*		2	3
2836	*cenea* main African stock	2706 H^yh	11	0	3	2	7
2890	*cenea* 2706 H^ch	2698 *antinorii* H^yh	49	19*		7	16
3050	yellow/*cenea* 2836 H^yH^c	hh	17	8	0	0	13

H^yh=yellow/*hippocoonides*.
hh=*hippocoonides*.
* Although in brood 2706 some of these were separable into heterozygotes for *hippocoonides* or *cenea*, others were not scorable as to which allelomorph they were carrying. In brood 2890 none of the insects were scorable for heterozygosity for *cenea* or *hippocoonides* and were identical in pattern with *antinorii* females.

scorable female offspring were of the *hippocoonides* pattern (i.e., *hippocoonides/niavioides*), two resembled *natalica* and are considered to be *natalica/niavioides* and two looked like yellow/*cenea* except that they were much more tawny. These are the presumed yellow/*natalica* hybrids. In wing pattern they resembled our wild "*natalica*" from Addis Ababa except that the black band on the hindwing was wider in this insect, and under ultraviolet light all these fluoresced slightly and to the same extent. Rather surprisingly the *natalica/niavioides* showed considerable fluorescence on the hind wings, but it seems that the scorability of this character is markedly affected by the presence or absence of brown scaling, the former tending to obscure the fluorescence, a feature which we noted in the tawny specimens of *proto-cenea*. The evidence therefore, on the whole, points to the *natalica*/yellow F_1 hybrids being intermediate between the parent forms and to *natalica* being dominant to *niavioides*.

Form *leighi* has not been found in Abyssinia, but brood 2816 (Table 5) in which a *leighi* from the "main African stock" was mated to an *antinorii* male, produced five females that closely resembled *leighi*, six which were *hippocoonides*-like, and one yellow female (yellow/*hippocoonides*) hybrid. We were uncertain for some time as to whether these five *leighi*-looking butterflies of brood 2816 were *leighi/hippocoonides* or *leighi*/yellow. However, broods 3196 and 3302 (Table 5) gave us butterflies which were clearly intermediate between *leighi* and yellow and this showed that the five females in 2816 were in fact *leighi/hippocoonides* with *leighi* fully dominant. The *leighi*/yellow hybrids were yellow butterflies, but with an orange subapical spot and orange spots on the hindwing border, both characteristic of *leighi*. In other words this hybrid was intermediate between the parent forms.

A further hybrid between the "main African stock" and yellow was obtained from brood 3075 (Table 6) (CLARKE and SHEPPARD 1960a), where a female bright *poultoni* (heterozygous for *hippocoonides*) was mated to a male of yellow/*hippocoonides* genotype. This produced four *hippocoonides*, three yellow/*hippo-*

TABLE 4

Broods giving details of natalica *and* trophonius *hybrids with yellow*

Brood no.	Form of mother and genotype where known	Origin of father and genotype where known	Males	Offspring: *natalica*/yellow females	Offspring: *trophonius*/yellow females	Offspring: *trophonius* females	Offspring: *natalica* females	Offspring: yellow/*cenea* females	Offspring: *hippocoonides* females
2725	*natalica* main African stock $H^{na}h$	wild *antinorii*	9	2	0	0	2	0	4
2741	*trophonius* main African stock $H^{T}H^{c}$	wild *antinorii*	13	0	5	3	0	3	0

$H^{na}h$ = *natalica/hippocoonides*.
$H^{T}H^{c}$ = *trophonius/cenea*.

TABLE 5

Broods giving details of leighi *hybrids with yellow*

Brood no.	Form of mother and genotype where known	Origin of father and genotype where known	Males	Offspring					
				Yellow/ *leighi* females	Recognizable heterozygote *leighi* / *cenea* females	*leighi* females	Yellow females	*hippocoonides* females	*cenea* females
2816	*leighi* main African stock H^Lh	wild *antinorii*	30	0	0	5	1	6	0
3006	*leighi* 2816 H^Lh	main African stock H^ch	47	0	1 H^LH^c	18	0	8	6
3196	yellow nonmimetic	3006 H^LH^c	10	1	0	1	1	0	3
3302	yellow/ *hippocoonides*	3006 H^Lh	14	2	0	3	1	6	0

H^ch = *cenea*.
H^LH^c = *leighi*/*cenea* or *leighi*.
H^Lh = *leighi*.

coonides, four bright *poultoni*/*hippocoonides* and six females resembling the *dorippoides* form of race *polytrophus*. Some of these were extreme, and others were nearer to bright *poultoni*. There were no *dorippoides* in the known ancestry of either the mother or father of brood 3075, and in this brood the form must, therefore, have been produced by a combination of the gene controlling yellow with that controlling bright *poultoni* (see CLARKE and SHEPPARD 1960a).

That a combination of the genes controlling pale *poultoni* and yellow can also produce *dorippoides* is shown by brood 3288 (Table 6). Here a yellow/*hippocoonides* female mated to a male heterozygous for *hippocoonides* and pale *poultoni*, gave two males, one *hippocoonides*, three pale *poultoni*, two yellow females resembling the yellow/*hippocoonides* hybrids but with more orange scaling, and five orange females closely resembling *dorippoides*, but again variable.

There is also evidence that *dorippoides* can be produced when modifiers for the male-like female form only are present, and not the major gene for yellow. Thus in brood 3295 (Table 6) a female pale *poultoni*/*hippocoonides*, which was also carrying part of the *antinorii* gene complex, when mated to a male homozygous for *hippocoonides*, produced three males, five pale *poultoni*, one *hippocoonides*, and two orange females resembling *dorippoides*, but with the color of pale *poultoni*.

Allelomorphism: Since we have shown that the genes controlling all the South African forms behave as allelomorphs (CLARKE and SHEPPARD 1959b) it follows that if any of them can be shown to be at the same locus as yellow then they are all allelomorphs of the male-like female form.

TABLE 6

Broods showing hybrids between yellow and bright and pale poultoni

Brood no.	Form of mother and genotype where known	Origin of father and genotype where known	Offspring							
			Males	Pale *poultoni* females	Bright *poultoni* females	Yellow/ *hippocoonides* females	*hippocoonides* females	*doripoides* females	*trophonius*/ pale *poultoni* recognizable heterozygote females	*trophonius* females
2927	*trophonius*/ yellow 2741	not known but must have carried pale *poultoni*	1	0	0	0	0	0	1	0
3075	bright *poultoni*	yellow/ *hippocoonides* 2890 $H^{y}h$ (see Table 3)	11	0	4	3	4	6 (bright orange)	0	0
3080	*trophonius*/ pale *poultoni* recognizable heterozygote 2927	main African stock *hh*	23	15	0	0	0	0	0	13
3288	yellow/ *hippocoonides* $H^{y}h$	$H^{pp}h$	2	3	0	2	1	5 (pale orange)	0	0
3295	pale *poultoni* 3080 (carrying part of *antinorii* gene complex)	*hh*	3	5	0	0	1	2 (pale orange)	0	0

hh = *hippocoonides*.
$H^{pp}h$ = pale *poultoni*.

In brood 2880 (Table 7), a *hippocoonides* female 2707 was mated to an F_1 male 2706, which could not have been homozygous for either *cenea* or yellow. The offspring of 2880 were nine males, four yellow/*hippocoonides* females and one *cenea* female. Again in brood 2929 (Table 7) a *hippocoonides* female 2711 was mated to a male 2725 which could not have been homozygous for either yellow or *natalica*. Brood 2929 produced 13 males, one yellow/*hippocoonides* female and six *natalica*/*hippocoonides* females. The fact that no *hippocoonides* or any other third phenotype were produced in either brood 2880 or 2929 strongly indicates that *cenea* and *natalica* are both alleles of yellow (see also 3050, Table 3).

Inheritance of tails: In Abyssinia the females and the males closely resemble each other in that both sexes have tails, whereas in the "main African stock" all the females are tailless. In the F_1 hybrids the females had either no tails or very short ones. In brood 2890 (Table 3) which was a backcross of an F_1 hybrid female

TABLE 7

Broods showing that cenea *and* natalica *are both allelomorphs of yellow*

Brood no.	Form of mother and genotype where known	Origin of father and genotype where known	Offspring: Males	Yellow/ *hippocoonides* females	*hippocoonides* females	*natalica* females	*cenea* females	*natalica*/ yellow females
2725	*natalica* main African stock $H^{na}h$	wild *antinorii*	9	0	4	2	0	2
2880	*hippocoonides* 2707 *hh*	2706 $H^{y}H^{c}$ (see Table 3)	9	4	0	0	1	0
2929	*hippocoonides* 2711 *hh*	2725 $H^{na}H^{y}$	13	1	0	6	0	0

$H^{na}h$ = *natalica*.
$H^{na}H^{y}$ = *natalica*/yellow.
hh = *hippocoonides*.
$H^{y}H^{c}$ = yellow/*cenea*.

of genotype *cenea/niavioides* mated to a male *antinorii* ($H^{y}h$), there was segregation for tail length not significantly differing from a 1:1 ratio—i.e., 20 butterflies had no tails, and 22 short, medium or full-length ones. Furthermore, the presence or absence of tails was independent of wing pattern (See Plate No. 9 for a tailed *cenea*). This suggests that tail length is controlled by a single major gene with modifiers, tailless (T) being semidominant to tailed (t). Confirmation of this was obtained from twelve F_2 matings, details of which are given in Table 8. It will be seen that there is a good approximation to the expected 3:1 segregation in respect of tailless to tailed in the combined families. The reduction in tail length in the Abyssinian hybrids is greater than that in the corresponding crosses using race *meriones* from Madagascar. The probable reason for this is mentioned later in this paper and discussed fully elsewhere (CLARKE and SHEPPARD 1960a,b).

To summarize, therefore:

(i) The yellow nonmimetic form *antinorii* is dominant to *niavioides*, *hippocoonides* and *cenea* and forms an intermediate when crossed with *natalica*.

(ii) *Cenea* and *natalica* are both dominant to *hippocoonides* (*niavioides*).

(iii) *Trophonius* is dominant to *hippocoonides* (*niavioides*) and semidominant to yellow.

(iv) *Leighi* forms an intermediate with yellow and is fully dominant to *hippocoonides* (*niavioides*).

(v) Either bright *poultoni* or pale *poultoni* in combination with yellow can produce an insect indistinguishable from form *dorippoides* of race *polytrophus*. Pale *dorippoides* has also been produced from a pale *poultoni*

TABLE 8

Inheritance of tails

Brood no.	Color of mother	Tailless		Tailed		Remarks
		Yellow	Not yellow	Yellow	Not yellow	
2859	Not yellow	0	11	0	9	.....
2871	yellow	1	1	0	0	.....
2872	yellow	3	0	0	0	.....
2878	yellow	1	0	1	0	.....
2879	Not yellow	5	10	2	2	1 yellow, lost
2880	Not yellow	2	1	2	0	.....
2885	Not yellow	1	1	1	0	.....
2887	yellow	4	1	1	2	.....
2922	yellow	0	1	0	0	.....
2929	Not yellow	1	2	0	1	3 not yellow, lost
2936	Not yellow	1	2	1	1	2 yellow and 2 not yellow lost
2937	Not yellow	0	1	0	1	1 not yellow lost
Totals			50		23	
2890	Not yellow	9	11	10	12	.....

Note: Broods 2859–2937 inclusive are F_2 matings. Brood 2890 is a backcross to *antinorii*.

which carried part of the *antinorii* gene complex, but which lacked the major gene for this pattern.

(vi) *Cenea* and *natalica* are both alleles of yellow and it follows from a consideration of the other evidence (CLARKE and SHEPPARD 1959b, 1960a) that all the forms we have investigated for allelism (with the possible exception of *proto-cenea*) behave as an allelomorphic series.

(vii) Tail length is controlled by a single major gene with modifiers, tailless (*T*) being semidominant to tailed (*t*). The locus is independent of that controlling wing pattern.

THE MADAGASCAN RACE MERIONES

In this race there is no polymorphism, all the females being nonmimetic, pale yellow and tailed like the males. We have obtained hybrids between *meriones*

(henceforth usually called yellow) and the South African mimetic forms *f. cenea*, *f. hippocoonides* and *f. trophonius*, and the South African nonmimetic forms *f. natalica* and *f. salaami*.

The autosomal inheritance of yellow in meriones

The reasoning which excluded sex-linkage in race *antinorii* also excludes it in race *meriones* (see Table 9). Thus broods 2433 and 2515 show that the gene for yellow cannot be on the Y chromosome and brood 2582 shows that it cannot be on the X. The gene for yellow is therefore autosomal.

Dominance relationships in crosses between race meriones *and race* cenea

The yellow/cenea *and yellow*/hippocoonides *hybrids:* In broods 2433 and 2515 (Table 9) a South African cenea heterozygous for *hippocoonides* was mated to a yellow male. The color and wing pattern of the resulting *cenea*/yellow and *hippocoonides*/yellow (Plate No. 5) hybrids were intermediate between the parent forms, but tended to resemble the mimics. In this respect they differed somewhat from the corresponding South African/*antinorii* hybrids, where the nonmimetic pattern was almost completely dominant in one brood and slightly less so in another. Using insects from Central Africa the dominance of yellow was almost complete in all broods. The absence of dominance in the *hippocoonides*/yellow hybrids is again demonstrated in the first backcross to *hippocoonides* (brood 2582, Table 11) where the *hippocoon*/yellow insects remained generally similar to those in the F_1, the *hippocoonides* characters being only slightly enhanced.

The yellow/trophonius *and yellow*/natalica *hybrids:* In the South African race of *P. dardanus* we have shown that *f. trophonius* and *f. natalica* form a heterozygote which is recognizable by the fawn color of the subapical spot, the rest of the wing color resembling *trophonius* (CLARKE and SHEPPARD 1959b). In brood 2562 (Table 10) we took such a heterozygous female and crossed her with a yellow male. The resulting F_1 female offspring were two *natalica*/yellow

TABLE 9

Autosomal inheritance of yellow in race meriones

Brood no.	Form of mother and genotype where known	Origin of father and genotype where known	Offspring		
			Males	*cenea*/ yellow females	*hippocoonides*/ yellow females
2433	*cenea* main African stock	*meriones* H^vH^v	17	5	8
2515	*cenea* main African stock	*meriones* H^vH^v	5	1	1
2582	yellow/*cenea* 2433	main African stock	7	7	4

hybrids and one *trophonius*/yellow hybrid. The former were again intermediate in color and wing pattern between the parents but veered towards the South African rather than the Madagascan form. The latter markedly resembled the imperfect mimic *lamborni* (a *trophonius*-like insect but with considerable yellow scaling) found in race *polytrophus*. However, the F_1 hybrids *trophonius*/yellow appear to be variable, since in brood 2450 a South African *salaami* female mated to a *meriones* male produced one insect in which the orange had spread to fill the subapical spot (Plate No. 8). This butterfly differed noticeably from the *lamborni*-like one. On backcrossing a 2562 male of genotype *trophonius*/yellow to a *natalica* female of the "main African stock", we found that the resultant *trophonius*-like insects were very similar to form *trophonius* of race *cenea* (brood 2714, Table 10). This might have been anticipated since in the F_1 *trophonius*/yellow hybrids the *trophonius* pattern was not altered to nearly the same extent as was *hippocoonides* in the *hippocoonides*/yellow and *cenea* in the *cenea*/yellow hybrids.

Allelomorphism

In brood 2582 (Table 11) we backcrossed a *cenea*/yellow F_1 hybrid to a male of "main African stock" which could not have carried yellow and was known not to be carrying *cenea*. The offspring were seven *cenea* and four yellow/*hippocoonides*, the latter being slightly less yellow than the F_1 hybrids. Brood 2582 suggests that yellow is an allelomorph of *cenea*, since no *hippocoonides* or any

TABLE 10

Broods showing hybrids between meriones *and main African stock*

Brood no.	Form of mother and genotype where known	Origin of father and genotype where known	Offspring						
			Males	*natalica*/yellow females	*trophonius*/yellow females	*trophonius* females	Heterozygote *natalica*/*trophonius* females	*cenea*/yellow females	*hippocoonides*/yellow females
2433	*cenea* main African stock	*meriones* $H^{u}H^{u}$	17	0	0	0	0	5	8
2450	*salaami* main African stock	*meriones* $H^{u}H^{u}$	0	0	1 (*salaami* like)	0	0	0	0
2515	*cenea* main African stock	*meriones* $H^{u}H^{u}$	5	0	0	0	0	1	1
2562	*natalica*/*trophonius* main African stock	*meriones* $H^{u}H^{u}$	2	2	1 (*lamborni* like)	0	0	0	0
2714	*natalica* main African stock	2562 $H^{T}H^{u}$	9	1	0	5	1	0	2

other third phenotype were produced. Further proof of allelomorphism is obtained from brood 2714 (Table 11) where a female *natalica* ($H^{na}h$) of "main African stock", known to be heterozygous for *hippocoonides* was mated to a male 2562. Brood 2714 produced four types of female, but again no *hippocoonides* or any other fifth phenotype. Broods 2582 and 2714 together, therefore, show that the yellow of *meriones* is an allelomorph of *cenea*, *natalica* and *trophonius*, since, if it were not, at least one other phenotype (probably *hippocoonides*-like) should have appeared.

Since the yellow of *meriones* and the yellow of *antinorii* are allelomorphs of the genes controlling the female forms in the "main African stock", it follows that the male-like forms in Madagascar and Abyssinia are also controlled by allelomorphs at the same locus.

Table 12 gives a complete list of the genetic symbols used in the three papers for the various genotypes.

TABLE 11

Allelomorphism in meriones *hybrids*

Brood no.	Form of mother and genotype where known	Origin of father and genotype where known	Males	Yellow/ *natalica* females	*natalica*/ *trophonius* females	*cenea* females	Yellow/ *hippocoonides* females	*trophonius* females
2582	*cenea*/yellow 2433	main African stock (not carrying *cenea*)	7	0	0	7	4	0
2714	*natalica* main African stock $H^{na}h$	H^TH^y 2562 (see Table 10)	9	1	1	0	2	5

TABLE 12

List of genetic symbols used in Parts I, II, and III

Phenotypes	Allelomorph	Genotypes
hippocoonides	h	hh
cenea	H^c	H^cH^c H^ch H^cH^{na}
natalica	H^{na}	$H^{na}H^{na}$ $H^{na}h$
trophonius	H^T	H^TH^T H^Th H^TH^c
leighi	H^L	H^LH^L H^LH^{na} H^LH^c H^Lh
salaami	..	H^LH^T
Imperfect *trophonius*	..	H^TH^{na}
Yellow	H^y	H^yH^y H^yh H^yH^c
Bright *poultoni*	H^{bp}	$H^{bp}H^{bp}$ $H^{bp}h$ $H^{pp}H^T$
Pale *poultoni*	H^{pp}	$H^{pp}H^{pp}$ $H^{pp}h$
Orange *cenea*	..	$H^{bp}H^c$
Buff *cenea*	..	$H^{pp}H^c$
**niobe*	H^{Ni}	$H^{Ni}H^{Ni}$ $H^{Ni}h$ H^TH^{Pl}
Red-brown *cenea*	..	$H^{Ni}H^c$
**planemoides*	H^{Pl}	$H^{Pl}H^{Pl}$ $H^{Pl}h$

* It is still possible that these genes are not allelomorphic with the others controlling color pattern.

Inheritance of tails

The tails of the F_1 hybrids tended to be shorter than those of the Madagascan parent, but there was great variability, and the dominance of tailless was much less marked than in the corresponding *antinorii* hybrids. This is probably because of the polymorphism in Abyssinia which will result in modifiers for shorter tails being selected for in the mimetic females. No such selection will be taking place in Madagascar where no mimics are present. (See CLARKE and SHEPPARD 1960a). To summarize, in the Madagascan crosses we find:

(i) The hybrids between *f. hippocoonides*, *f. natalica* and *f. cenea* from race *cenea* and the yellow nonmimetic form *meriones* have intermediate wing patterns. This is in sharp contrast to the corresponding hybrids with race *antinorii* (excepting some insects in one brood) which are very similar in appearance to the yellow form.

(ii) The yellow of *meriones* behaves as an allelomorph of *cenea*, *natalica* and *trophonius*, and it follows that it is also an allelomorph of the yellow of *antinorii*.

(iii) The tail length of the Madagascan hybrids tends to be less than that of the *meriones* parent but is greater and more variable than in the corresponding *antinorii* crosses.

This concludes our survey of the formal genetics of *Papilio dardanus*. From a consideration of the three papers it will be recognized that each form that we have investigated for allelism (with the possible exception of *proto-cenea* in race *polytrophus*) is controlled by a gene which behaves as one of an allelomorphic series. In fact, however, it seems more probable that we are dealing with a series of closely linked genes resulting in the formation of a supergene, (see CLARKE and SHEPPARD 1960d).

The evolution of dominance and the method by which the mimicry may have evolved are also dealt with elsewhere (CLARKE and SHEPPARD 1960b,c) as is the technique of breeding the insect in this country, (CLARKE and SHEPPARD 1959a).

SUMMARY

1. In the three parts of this paper are discussed the genetics of most of the female forms of the mimetic butterfly *P. dardanus*.

2. The genes controlling the pattern of the nonmimetic male-like females, *natalica*, *leighi*, bright *poultoni*, pale *poultoni* and the mimics *hippocoonides*, *cenea* and *trophonius* are sex-limited in effect and behave as an autosomal multiple allelomorphic series.

3. The genes controlling the mimics *planemoides* and *niobe* also probably belong to this series but sufficient information is not available at this point.

4. It is now known that the very rare form *salaami* from South Africa is a heterozygote between *trophonius* and *leighi*.

5. The genetics of *proto-cenea* are not yet clear.

6. The autosomal locus controlling the presence or absence of tails on the hindwings is independent of that controlling pattern. The absence of tails, a characteristic feature of females of all races, except those from Abyssinia, Madagascar and Grand Comoro which are tailed, is sex-limited to the females.

7. The dominance relationships between the forms are complex. However, dominance is absent more frequently when the forms concerned are allopatric than when they are sympatric. This suggests that dominance between sympatric forms has been evolved.

8. The average length of the tails of the Abyssinian females, particularly the mimetic ones, is less than that of the males or of the Madagascan females. This is apparently due to the presence of modifiers and accounts for the difference in the degree of dominance of the tailless condition in F_1 hybrids depending on whether an Abyssinian or Madagascan insect is used as the tailed parent.

9. On introducing a gene controlling a mimetic pattern into the gene complex or part of the gene complex of a race that does not possess it, the mimetic pattern tends to become imperfect. This suggests that the present day mimics have been built up from forms showing a less perfect resemblance to the model, as the result of the selection of modifiers.

10. The color patterns of the allopatric forms *hippocoonides*, *hippocoon* and *niavioides*, which mimic various subspecies of *Amauris niavius*, differ in the modifiers present and not by major genes. This is apparently also true of the difference between *trophonius* and *trophonissa* which forms mimic *Danaus chrysippus*.

11. *Hippocoonides* and its modifications are recessive to all the forms sympatric with them.

12. Two new forms from Abyssinia are reported, one has the color pattern of *cenea* and one of *natalica*. Both have tails as do all the other females of this race.

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CENTRIC FRAGMENTS AND POLLEN-PART MUTATION OF INCOMPATIBILITY ALLELES IN PETUNIA[1]

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THE incompatibility (*S*) alleles of flowering plants have two functions—one determining pollen phenotype, the other shaping the stylar phenotype. That these two functions can be separated as the result of mutation was observed by LEWIS (1949), who obtained self-fertile mutants in which an *S* allele's pollen activity alone had been altered. Similar pollen-part mutants have predominated among spontaneous and radiation-induced mutants obtained in subsequent investigations (LEWIS and CROWE 1954; PANDEY 1956; BREWBAKER and SHAPIRO 1959).

Self-fertility is known to appear in many self-incompatible species as the result of induced tetraploidy (LEWIS 1947; BREWBAKER 1954). This breakdown of incompatibility is based on a novel *S* gene interaction termed "competition" which follows when two different alleles occur in the same pollen grain. Such interaction characterizes all *S* allele heterozygotes tested in *Petunia inflata* Fries, the species employed in this study (BREWBAKER and SHAPIRO, unpublished data).

Centric chromosome fragments occur widely in plants, often as euchromatic and genetically inert "B" chromosomes. Other supernumeraries evidently are derived by more or less recent fragmentation from the standard "A" genome (GRUN 1959; MUNTZING 1958). Irradiation is a notoriously effective tool for the induction of such fragmentation.

Centric fragments, competition and pollen-part change are linked in the present study to afford a cytogenetic basis for pollen-part mutation and to suggest a possible reason for the establishment and distribution of fragments in some flowering plants.

MATERIALS AND METHODS

All mutants were obtained following irradiation of a highly self-incompatible seed stock of *Petunia inflata* Fries ($2n = 14$). Incompatible matings to screen for *S* mutants were made exclusively on unirradiated pistils. Testers included *S* allele homozygotes of garden hybrid origin to which the alleles had been transferred from *P. inflata*. Mutant seeds were obtained only rarely from homozygotes, while they were recovered frequently from the heterozygotes, particularly from buds irradiated shortly prior to meiosis (BREWBAKER and SHAPIRO 1959).

The 3100 pollinations from which data are summarized here were made in

[1] Research carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.

bee-proof greenhouses. Flowers were emasculated in late bud stage on all self-fertile and most self-sterile plants. Fertile crosses averaged over 150 seeds per pollination, while incompatible matings averaged 0.024 seed/pollination using homozygous male testers and 0.204 seed/pollination using heterozygotes. Cytological observations were made on root-tip smears stained with aceto-orcein and on PMC's and pollen tubes stained with propionocarmine.

RESULTS

General survey of S *mutants*

At the time of writing, 68 plants had been grown successfully from X_1 "mutant" seed of *P. inflata* and identified genotypically. Eighteen of these X_1 plants were derived from homozygous self-incompatible (SI) parents, and each of these found to be of the same genotype as its parent, i.e., nonmutant.

Similarly, 20 of the 50 X_1 plants derived from heterozygous parents were self-incompatible, reacting phenotypically just as the parents. Plants of this nonmutant genotype have been found in all studies of *S* gene mutation. Among nine additional X_1 mutants derived from heterozygotes, four were female sterile and five were both female and male sterile. This sterility was exceedingly variable and associated with *S* gene change in only two instances. The remaining 21 self-fertile X_1 mutants included 15 in which the pollen activity alone had been altered.

Pollen-part mutants

Each of the 15 self-fertile X_1 plants categorized as a pollen-part mutant was fully self-fertile (SF), universally cross-fertile as male (F as ♂) and identical to its S_1S_2 parent as female (S_1S_2 as ♀). None of the mutant plants reacted as if homozygous for *S* alleles, a significant discrepancy from the expected 50 percent. The plants chosen for cytogenetic studies to be reported were obtained by X-irradiation (500 or 1000r) of the clone S_1S_2 prior to premeiotic interphase, and each derived from a separate mating. All progenies were self-pollinated, crossed as females to S_1S_1, S_2S_2, S_5S_5, S_1S_2 and S_1S_5 testers and crossed as males to appropriate tester genotypes. Pollen viability *in vitro* of all plants exceeded 75 percent, comparing favorably with the controls. Cytogenetic studies are reported here for five of the pollen-part mutants and their progenies.

(1) X9-4: Sixteen plants were grown from the cross of pollen-part mutant X9-4 (SF, F as ♂, S_1S_2 as ♀) with the homozygote S_5S_5 (Table 1). Seven plants were SI, segregating six S_1S_5 and one S_2S_5, indicating that X9-4 carried non-mutant chromosomes bearing S_1 and S_2. A unique *S* genotype, $S_1S_2S_5$ as ♀, was obtained among the nine SF plants. Six of these plants were universally cross-fertile as males but cross-sterile as females to S_1S_1, S_2S_2, S_5S_5, S_1S_2, S_1S_5, and S_2S_5 testers, and could be considered only as of the triallelic $S_1S_2S_5$ genotype as female. The three remaining SF plants reacted as S_2S_5 females.

A study of the mitotic chromosomes of six of the self-fertile plants (four $S_1S_2S_5$, two S_2S_5) revealed the presence in each of a small centric fragment (Figures 1–4). No such fragments could be observed in the three self-sterile plants studied. The fragments showed no irregularities in behavior, and could be discerned readily during meiosis and pollen tube mitosis (Figures 1–4).

TABLE 1

Incompatibility reactions and cytology of progenies from pollen-part mutants

Family	Number of plants	Self-fertility	Incompatibility reaction as: male	Incompatibility reaction as: female	Cytology
X9-4 × S_5S_5	6	SI*	S_1S_5	S_1S_5	14 (3 plts.)
	1	SI*	S_2S_5	S_2S_5	.....
	6	SF*	F*	$S_1S_2S_5$	14 + frag* (4 plts.)
	3	SF*	F*	S_2S_5	14 + frag (2 plts.)
X10-1 × S_5S_5	4	SI	S_1S_5	S_1S_5	14 (1 plt.)
	12	SF	F	$S_1S_2S_5$	14 + frag (4 plts.)
	1	SF	F	S_1S_5	.....
	1	SF	F	S_2S_5	.....
X18-1 × S_5S_5	4	SI	S_1S_5	S_1S_5	14 (1 plt.)
	3	SI	S_2S_5	S_2S_5	14 (1 plt.)
	5	SF	F	$S_1S_2S_5$	14 + frag (1 plt.)
	1	SF	F	S_2S_5	14 + frag
	1	SF	F	F	.....
X19–1 selfed	12	SF	F	S_1S_2	14 + frag (2 plts.)
S_1S_2 × X19-1	13	SF	F	S_1S_2	.....
X19-1 × S_5S_5	8	SI	S_2S_5	S_2S_5	14 (3 plts.)
	5	SF	F	$S_1S_2S_5$	14 + frag (3 plts.)
	1	SF	F	S_5S_5	14 + frag
	1	SF	F	F	14 + frag
X22-2 × S_5S_5	2	SI	S_1S_5	S_1S_5	.....
	6	SI	S_2S_5	S_2S_5	.....
	2	SF	F	S_1S_5	.....
	1	SF	F	$S_1S_2S_5$	.....

* SI = self-incompatible, SF = self-fertile, F = fertile, frag = centric fragment.

The results of this and succeeding families are explained satisfactorily by the hypothesis that a fragment carrying an *S* allele was induced by radiation. As a consequence of competition interaction, fragment-bearing heterogenic pollen would be uninhibited in pollen tube growth, accounting for the observed self-fertility. The cross X9-4 (S_1S_2 + fragment S_2) × S_5S_5 would be expected on this hypothesis to produce ¼ S_1S_5: ¼ S_2S_5: ¼ S_1S_5 + frag S_2: ¼ S_2S_5 + frag S_2, an expectation with which the observed data were in accord.

(2) X10-1: Twenty plants were grown from the cross X10-1 × S_5S_5, of which 18 were classified genotypically (Table 1). Rather too much variability was observed among the 780 selfs and crosses made in this family to permit unequivocal classification of all plants, since many plants were poor seed setters. Four of those classified were SI, S_1S_5, while the remaining 14 were SF. The SF, F as ♂ and $S_1S_2S_5$ as ♀ class appeared again in this family and comprised 12 plants. Two other SF plants were classified as S_1S_5 as ♀ and S_2S_5 as ♀, respectively.

Cytological examination again revealed a small centric fragment in four of the SF plants and none in the SI plant tested. In this case, it was possible also to test the SF parent, X10-1, and rewarding to find the fragment present as expected. It is suggested that X10-1 was S_1S_1 + fragment S_2. The two unexpected SF geno-

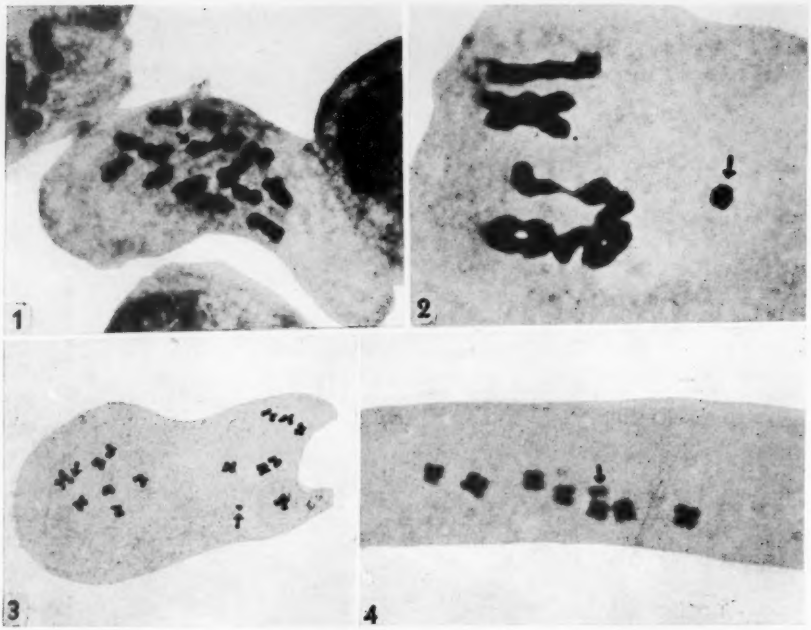

FIGURE 1–4.—Cytological behavior of the centric fragment. FIGURE 1.—Somatic chromosomes of a mutant showing 14 chromosomes and the centric fragment in a root-tip squash. FIGURE 2.—First metaphase in a PMC, showing seven bivalents and the fragment. FIGURE 3.—First anaphase groupings, showing seven chromosomes plus the fragment on each pole. FIGURE 4.—Pollen tube mitosis showing seven chromosomes and the fragment.

types deserve further test although the S_1S_5 as ♀ could be expected as a result of crossing over.

(3) X18-1: Fourteen plants were studied from the cross X18-1 × S_5S_5, of which seven were SI, including four S_1S_5 and three S_2S_5 (Table 1). The seven SF plants comprised five $S_1S_2S_5$ as ♀ and one S_2S_5 as ♀. An additional SF plant set seed reciprocally with every tester genotype (including homozygotes) and was classified as SF, F as ♀. Similar plants have been obtained as direct X-mutants or segregants on six separate occasions and are the subject of continued study.

Cytological studies revealed single fragments in each of two SF plants tested as well as in the parent clone, while two tested SI plants presented the normal $2n = 14$ complement. The data are satisfactorily explained by assuming that X18-1 was S_1S_2 + fragment S_2.

(4) X19-1: Three families were grown from X19-1 (Table 1). The selfed family comprised plants of only one *S* phenotype—that being the same as the parent X19-1 (SF, F as ♂, S_1S_2 as ♀). Similarly the backcross of X19-1 as male to its parent produced plants of only one *S* phenotype, again that of X19-1. Seventeen plants were grown from the cross X19-1 × S_5S_5, and these segregated into

four groups. Ten of these were SI, of which the eight tested plants were S_2S_5. $S_1S_2S_5$ plants again prevailed among the SF group, while two additional SF plants —the subject of some 140 crosses—were of the unexpected phenotypes (1) SF, F as ♂, F as ♀ and (2) SF, F as ♂ and S_5S_5 as ♀.

Fragments were observed in each of the seven tested SF plants of these three families, as well as in the parent X19-1. None of the three SI plants tested carried fragments.

(5) X22-2: Plant X22-2 was a self-fertile pollen-part mutant differing from those considered previously in the unreliability of its behavior as a female. In a first set of pollinations X22-2 was SF, F as ♂ and S_1S_2 as ♀; in a second season, it was SF, F as ♂ and F as ♀ (perhaps affording a key to the origin of this latter phenotype). X22-2 represented an odd family; its sibs from the same mutant capsule included a prostrate sterile dwarf and three pollen-part mutant plants, of which one was SF, F as ♂ and S_1S_2 as ♀, one SF, F as ♂ and F as ♀ and one female sterile, F as ♂.

The 11 plants grown from X22-2 × S_5S_5 were rather more conventional in behavior, and similar to families reported previously (Table 1). The results are explained satisfactorily by assuming that X22-2, which carried a fragment, was genotypically S_1S_2 + fragment S_1.

DISCUSSION

The five pollen-part mutants studied have been shown to be self-fertile by reason of their inclusion of *S* gene bearing fragment chromosomes and production of the uninhibited heterogenic competition pollen type, S_1S_2 (a genotype which has also been studied in 3n and 4n lines). The karyotypes proposed for the five mutants were as follows:

X10-1	S_1S_1 + fragment S_2
X19-1	S_2S_2 + fragment S_1
X22-2	S_1S_2 + fragment S_1
X9-4 and X18-1	S_1S_2 + fragment S_2

All four combinations are thus represented, and the expected 1:1 ratio of homozygous to heterozygous progeny satisfied. The fragments were of relatively constant size (about ⅕ length of chromosome) and with median centromeres. They clearly did not affect pollen viability or activity, e.g., the expected 1:1 segregation of seven and seven + fragment pollen types was realized satisfactorily by the test-cross segregation of 36 SI:40 SF plants.

No cytological studies have been reported previously for pollen-part mutants, nor have the extremely elucidative homozygotes been employed as testers for X_1 plants. LEWIS (1949) proposed that independent loss of pollen activity proved that the *S* gene was bipartite, having independently mutable pollen and stylar components. Subsequent studies have concurred without lending particular support to this hypothesis (LEWIS and CROWE 1954; PANDEY 1956). Some if not all of the pollen-part mutants of these other plants (Oenothera, Prunus and Trifolium spp.) can be satisfactorily explained on a fragment or *S* gene translocation basis.

An exception is the class of nine X_1 plants concluded by PANDEY (1956) to be pollen-part mutant homozygotes. These nine plants could also be interpreted as *S* gene deletions which are lethal when homozygous, placing them more realistically with their sibs, in which *S* deletion prevailed (28 of 29 plants classified).

The origin of self-fertility in a self-incompatible species as the result of the addition of a chromosome fragment arouses speculation concerning the origin of centric fragments in general. *S* gene bearing fragments have the immediate advantage, when competition occurs, of indifference to an incompatibility screen. They could be expected to spread widely in a self-sterile population, since competition grains carrying fragments would be universally uninhibited. Self-fertility *per se* would confer a considerable survival advantage to the fragment and assist in its establishment in the species. Subsequent *S* gene loss (heterochromatization) by the fragment would not be entirely unexpected if inbreeding depression later endowed it with selective disadvantage.

SUMMARY

Genetic analyses of five pollen-part incompatibility allele mutants in Petunia indicated that all resulted from the addition of an *S* gene bearing centric fragment to the normal genome. Fragment-carrying heterogenic pollen grains were uninhibited in all matings as a result of competition interaction (observed similarly for heterogenic diploid grains of the corresponding tetraploids). It is suggested that the induction of self-fertility may provide a selective mechanism for the establishment and distribution of supernumerary centric fragments in a species.

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THE EVOLUTIONARY DYNAMICS OF A POLYMORPHISM IN THE HOUSE MOUSE[1]

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OVER the past several years DUNN and his collaborators have shown that most wild populations of *Mus musculus* in the United States are polymorphic for variant alleles at the *T* (Brachy, short tail) locus (DUNN and SUCKLING 1956; DUNN 1957). A mutant *t* allele at this locus has been identified in each of 16 different populations, and only one population thus far tested has been shown with any degree of certainty to be free of such alleles.

This widespread polymorphism has several remarkable features which make it unlike other polymorphic systems so far studied. First, of 16 alleles from wild populations, 13 are unconditional prenatal lethals when homozygous ($t^{w1, 3, 4, 5, 6, 10, 11, 12, 13, 14, 15, 16, 17}$). The remaining three ($t^{w2, 7, 8}$) although viable when homozygous, are completely male sterile. Thus, even if it is supposed that the viability of homozygotes for these latter alleles is as high as for normal homozygotes, and that the fertility of homozygous females is normal, the t^w alleles are at least semilethal in a genetic sense. In the ordinary course of events such alleles should be reduced to an extremely low frequency in a population unless some other mechanism countervened to maintain them.

The second unusual aspect of this polymorphism is the abnormal ratio of + and *t* gametes in the effective sperm pool of heterozygous males ($+/t^w$). About 95 percent of the offspring of $+/t^w$ males carry the t^w allele while only five percent carry the + allele from the father. Determinations of this ratio can be made by utilizing the dominant allele *T*. Heterozygotes $T/+$ have short ("Brachy") tails, heterozygotes T/t^w are completely tailless, and heterozygotes $t^w/+$ have normal tails (CHESLEY and DUNN 1936). Test matings of two sorts can be made.

A.	♂ T/t^w	×	♀ $+/+$
	tailless		normal
B.	♂ $+/t^w$	×	♀ $T/+$
	normal		Brachy

Normal Mendelian segregation would result in equal numbers of Brachy ($T/+$) and normal ($t^w/+$) offspring from type A matings. From type B matings normal segregation would produce equal numbers of $+/+$ (normal), $+/t^w$ (normal), $T/+$ (Brachy) and T/t^w (tailless) offspring. The ratio of Brachy to tailless offspring reveals the ratio of + to *t* in the effective sperm pool. Such matings

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of males heterozygous for a *t* allele from a wild population always produce a large excess of offspring carrying t^w. As the results of both type A and type B matings have been in complete accord with each other when tested, most of the characterization of segregation ratios has been carried out with the more efficient type A mating system (DUNN 1957). Table 1 shows the pooled results of all type A tests so far run on alleles of wild origin. The great excess of t^w gametes in the effective pool of sperm from heterozygous males is striking. These results cannot be ascribed to intrauterine selection against Brachy embryos for two reasons. First the results of type B matings are the same, and second, the cross of T/t^w females by normal males produces Brachy and normal offspring in equal numbers (Table 1), DUNN 1957.

Thus, the mutant alleles, although lethal or semilethal in homozygotes, are present in great excess in the effective sperm of heterozygous males and in normal proportions in the ova of heterozygous females.

Finally, there is some evidence of a greater fitness of heterozygous males relative to homozygous normal males (DUNN, BEASLEY and TINKER 1958). This is as yet preliminary, involving only a single allele, t^{w11}, and based upon a small number of animals. Taken at face value, the data show the heterozygous males $+/t^{w11}$ to have a fitness of 1.76 times that of homozygous normal males. This estimate is based upon only 53 individuals, however, and should not be given too much weight. In the same experiment only 19 females could be successfully tested and these showed no evidence of differential fitness. Nevertheless, the suspicion of

TABLE 1

Pooled segregation ratios of tailless males, heterozygous for t *alleles, each derived from a different wild population*

	Offspring classified at birth			
1 Males tested*	2 Normal $(+/t^w)$	3 Brachy $+/T$	4 Total	5 Ratio of t^w
T/t^{w1}	325	38	363	.895
T/t^{w2}	325	17	342	.950
T/t^{w3}	355	3	358	.992
T/t^{w4}	187	5	192	.974
T/t^{w5}	479	30	509	.941
T/t^{w6}	230	4	234	.983
T/t^{w7}	214	16	230	.930
T/t^{w8}	178	25	203	.876
T/t^{w10}	240	7	247	.972
T/t^{w11}	518	19	537	.965
T/t^{w12}	412	19	431	.956
T/t^{w13}	502	11	513	.979
T/t^{w14}	392	21	413	.949
T/t^{w15}	415	41	456	.922
T/t^{w16}	107	6	113	.947
T/t^{w17}	336	1	337	.998
All males	5,215	263	5,478	.952
All females T/t^w	199	188	387	.517

* Fives males of each allelic type were tested by mating with wild type +/+ females.

heterosis from the male data suggests that an intensive study of this aspect of the problem would be worthwhile.

Maintenance of the polymorphism

In the light of our present knowledge of the action of the t^w alleles it would seem that the polymorphism in nature is maintained by a balance between selective elimination of the mutant alleles in homozygous condition and the replenishment of these alleles by the extremely aberrant segregation ratio in heterozygous males. In addition, there is the possibility of heterosis as a balancing force.

There are two models that may be constructed for the evolution of the polymorphism, one deterministic and the other stochastic, i.e., involving chance processes. The deterministic model assumes that natural populations have very large breeding size, effectively infinite, and that the opposing forces of selection and abnormal segregation are entirely responsible for the state of the polymorphism in any population. The stochastic model, on the other hand, rejects the assumption of infinite population size and allows for the effect of random genetic drift and the probablistic nature of selection. It is this latter model which is certainly closer to the real situation and, as we will presently show, more nearly explains what is observed in nature.

The general stochastic theory of population genetics is, as is well known, due to SEWALL WRIGHT. He has shown, in a series of papers too numerous to cite, that finite population size leads to fixation of alleles at rates which depend on population size, migration, mutation, selection, etc. The most general summary of this theory is to be found in WRIGHT (1949), and the results of the present paper are in accord with his general predictions.

The deterministic model

The first algebraic solution to the problem of the balance between selection and abnormal segregation ratio was given by PROUT (1943). This solution placed no restriction on the relative fitnesses of the genotypes but turns out to be incorrect when the segregation ratio is not equal in the two sexes. Because of this latter restriction, PROUT's solution is not applicable to the problem at hand.

BRUCK (1957) has given a series of expressions for the equilibrium gene frequency and frequency of heterozygotes for the case of a recessive lethal with abnormal segregation in one sex only. This solution is considerably closer to the situation in Mus and would be completely applicable except for the few nonlethal alleles and the fact that fitness values may be different in the two sexes. As a first approximation to the natural situation, however, BRUCK's model is quite satisfactory. Letting

m = proportion of t^w gametes in the effective sperm pool

$\hat{p}$ = equilibrium frequency of + alleles among adults

and

$\hat{h}$ = equilibrium frequency of heterozygotes among adults

then

$$(1)\quad \hat{p} = 1/2 - \frac{\sqrt{m(1-m)}}{2m}$$

and

$$(2)\quad \hat{h} = 2(1-p) = 1 - \sqrt{\frac{1-m}{m}}$$

Referring to Table 1, the lowest value of m is that for t^{w8} ($m = .876$), and the highest is for t^{w17} ($m = .998$). The average of all ratios is .952. Substituting these three values in equations (1) and (2) above we find

t^{w8}: $\hat{p} = .788$ $\hat{h} = .624$
t^{w17}: $\hat{p} = .522$ $\hat{h} = .956$
average: $\hat{p} = .614$ $\hat{h} = .772$

Thus, between 60 and 95 percent of the adults in natural populations should be heterozygous for a mutant t allele. As we will discuss in a later section, this is much too high a frequency to correspond to what is actually found in nature. If, in addition, there is heterosis, the difficulty is exacerbated so that, in a sense, the deterministic solution proves too much.

The stochastic model

The deterministic model does not take into account the inbreeding which results from the restricted breeding size of Mus populations.

Although there are no direct observations from which the effective size of a breeding unit in this species can be estimated, the indications are that it is limited. The home ranges in a few populations studied appear to be small (review in BLAIR 1953); there are differences among local groups in the frequencies of different phenotypic characters (DUNN, BEASLEY and TINKER 1960; DEOL 1958); gene frequency observations show that different local groups are polymorphic for different alleles at one locus (t alleles) (DUNN 1957). The species population appears to consist of many partially separated breeding groups, each small in relation to the whole population. Although some local groups reach high densities and numbers (SOUTHWICK 1958), they probably pass through small population minima, and it is these which exercise a controlling effect on the evolution of gene frequencies. For these reasons, a useful approach in the construction of models is to test the effects on gene frequencies of small effective size of the breeding unit.

One method of dealing with the interaction of selection, segregation abnormality, and restricted population size is the construction of a stochastic model of population dynamics. Such a stochastic model may be analysed either by the algebraic theory of stochastic matrices (FELLER 1950) or by a *Monte Carlo* procedure. We have chosen the latter method as being the more efficient for our essentially exploratory study. In a Monte Carlo procedure, a mechanical or numerical analogue of a real population is formed and the rules by which the analogue population reproduces itself are made to conform with genetic rules of meiosis, fertilization, and selection. In addition chance plays a role in determining whether a given individual will survive and leave offspring. The mechanisms for Monte Carlo sampling may be beads mixed in an urn, numbers chosen from a table of random numbers, cards chosen from a shuffled pack, etc. The first sug-

gestion that such procedures might be useful for the *t* allele problem was made to one of us by PROF. GERT BONNIER in 1956. PROF. BONNIER saw clearly that finite population size must lead to eventual fixation of the normal alleles in contrast to the prediction of the deterministic model. He further suggested that "migrations of single but vigorous $+/t$ males" might be responsible for maintenance of the polymorphism. As will be shown later, we reach essentially the same conclusions.

As an analogue to a natural population we have used the IBM 650 digital computer. The scheme of the Monte Carlo program is shown in Figure 1. A two-digit number represents each individual. Because of the nature of the computer, it was convenient to let 88 stand for $+/+$, 89 for $+/t$, and 99 for t/t genotypes. N♂ males and N♀ females are initially loaded into the machine in any desired genotypic combination. A separate sperm and ovum pool are calculated and stored. As the number of gametes is very large compared with the number of adults, the gametic pools are computed deterministically, using predetermined values of m♂ and m♀, the segregation ratios for males and females. What is actually stored are the proportions p♂ and p♀ of two gametes in each pool. Next, a random number is generated by the method of BOFINGER and BOFINGER (1958). This number which lies between zero and one determines whether an eight or a nine gamete is chosen from the sperm pool. If the number is less than or equal

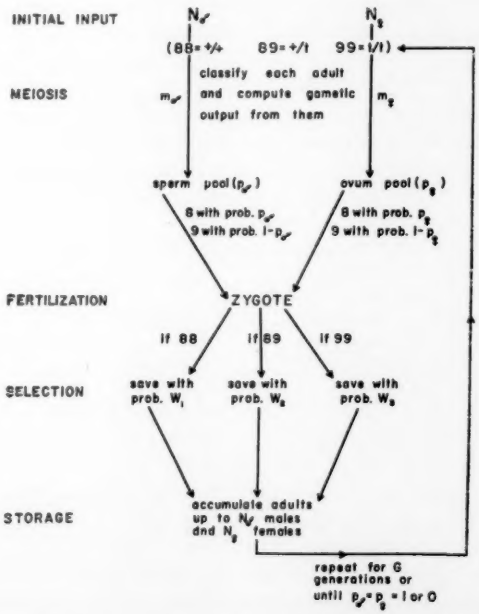

FIGURE 1.—Schematic representation of the Monte Carlo program for the 650 computer. Each stage is more fully explained in the text.

to $p\delta$ an eight is chosen, while if it is greater than $p\delta$ a nine is chosen. The same procedure is repeated for the pool of ova with the result that a zygote is formed which may be an 88, 89, or 99. The zygote so formed is classified as to its genotype in preparation for the selection phase. A new number is generated and if this number is less than or equal to W, the fitness of the particular genotype of the zygote just formed, the individual is saved and stored as an adult of the next generation. Otherwise, it is discarded. The processes of gamete choice, fertilization, and selection are repeated until $N\delta$ males have been saved and stored. Obviously this means that some larger number of males must be generated since selection discards a certain proportion of generated individuals. If necessary, the male fitness values are now replaced by female fitnesses and the entire process is repeated until $N\varphi$ females have been saved, whereupon the saved males and females become the adults of the next generation, replacing the input males and females. The entire cycle from adults to adults is repeated until a predetermined number of generations has passed, or until the population is completely homozygous, whichever occurs first. During the computation the following information is printed each generation:

G: the generation number
$D\delta$: number of $+/+$ adult male parents
$H\delta$: number of $+/t$ adult male parents
$R\delta$: number of t/t adult male parents
$D\varphi$: number of $+/+$ adult female parents
$H\varphi$: number of $+/t$ adult female parents
$R\varphi$: number of t/t adult female parents
p: frequency of $+$ allele among parental adults
$p\delta$: frequency of $+$allele in sperm produced by parents
$p\varphi$: frequency of $+$allele in ova produced by parents.

The parameters that are assigned before the program is run are:

$N\delta$: number of adult males maintained
$N\varphi$: number of adult females maintained
$W1\delta$: fitness of $+/+$ males
$W2\delta$: fitness of $+/t$ males
$W3\delta$: fitness of t/t males
$W1\varphi$: fitness of $+/+$ females
$W2\varphi$: fitness of $+/t$ females
$W3\varphi$: fitness of t/t females
$m\delta$: proportion of t gametes from a heterozygous male
$m\varphi$: proportion of t gametes from a heterozygous female
Gmax: maximum number of generations for run.

In addition, the exact genotypic composition of the initial generation must be specified. About 1.5 seconds are required to compute each generation for the parameter sets used in this study.

The general approach was to make a large number of runs with identical parameter sets and input populations in order to get an idea of the distribution of results. No two runs are identical because of the random effects introduced. Thus, making 100 runs with the same set of parameters is equivalent to setting up 100

experimental populations all with the same forces of selection, random drift and segregation. It is then possible to deal with means and variances just as in experiments with real populations.

Results of the Monte Carlo trials

Table 2 shows the various sets of parameters used and the number of runs for each parameter set. Because of the lack of any decisive information to the contrary, the fitnesses of males and females were assumed to be the same; the homozygous normal and heterozygotes were assumed to have the same fitness, and the female segregation ratio was assumed to be normal. The data so far available validate the last assumption.

A. *The large populations:* Sets 1 and 2 were not run more than a few times because it became evident that they were behaving in a virtually deterministic manner. The effective population sizes of 50 and 20, respectively, for these two sets were too high for any appreciable random effect to be felt. Examination of the output from each generation showed that it was extremely unlikely that populations with these parameters would reach fixation before many hundreds of generations. The pertinent statistics for parameter sets 1 and 2 are given in Table 3. These are based upon all generations except the first two in each run. The first two generations are eliminated in each case to avoid the effect of the initially imposed frequencies. As the table shows, the observed means are very close to the theoretical value of $p = .615$ from BRUCK's equation. It should be noted, however, that the confidence intervals for p do not quite overlap the theoretical value. Both observed values are smaller than theoretical, the small population deviating more than the large. As will be discussed below, this effect is even stronger in the still smaller populations. As expected, the variance and range of values for set 1 is less than for set 2, but even in set 2 the closest approach to homozygosis was six $+/+$ males, four $+/t$ males, and ten $+/+$ females. Because

TABLE 2

Parameter sets for Monte Carlo trials

Set	$N\sigma^{\prime}$	$N\varphi$	$m\sigma^{\prime}$	G max.	Initial composition: Male* $+/+$	Male* $+/t$	Male* t/t	Female† $+/+$	Female† $+/t$	Female† t/t	Number of runs
1	25	25	.95	50	6	13	6	6	13	6	3
2	10	10	.95	200	2	6	2	2	6	2	3
3	4	4	.95	200	1	2	1	1	2	1	50
4	4	4	.90	200	1	2	1	1	2	1	50
5	2	6	.95	200	0	2	0	2	4	0	101
6	2	6	.95	100	1	1	0	6	0	0	119
7	2	6	.95	100	2	0	0	5	1	0	30
8	2	6	.98	200	0	2	0	2	4	0	56
9	2	6	.98	100	1	1	0	6	0	0	58
10	2	6	.90	200	0	2	0	2	4	0	56

* In all cases the fitness values for males and females are $W_1 = W_2 = 1$, $W_3 = 0$.
† Female segregation is normal ($m\varphi = .50$).

TABLE 3

Statistics calculated from parameter sets 1 and 2 of the Monte Carlo trials

Set	Total generations	$\bar{p}$	$\bar{q}$	σ_p^2	σ_p	$\bar{\sigma}_p$	95 percent conf. int. for p
1	144	.6042	.3958	.00296	.0544	.00453	.5953–.6131
2	594	.5941	.4069	.00591	.0769	.00316	.5879–.6003

	Extremes						
	Female		Male				
Set	+/+	+/t	+/+	+/t	p	q	h
1	14	11	17	8	.81	.19	.38
	1	24	0	25	.51	.49	.98
2	6	4	10	0	.90	.10	.20
	0	10	0	10	.50	.50	1.00

of the segregation ratio in males, this high frequency of the + allele was immediately reduced in the next generation. It is this sort of result which led us to abandon these parameter sets and consider smaller population sizes.

B. *Small populations:* Because of the very large number of generations and runs, only a sample of the data has been analyzed for parameter sets 3–10. In all that follows, statistics are based on every tenth generation in each run.

For any given parameter set all populations (runs) were started with the same composition. After one generation, however, because of the random events in the model, all populations no longer have the same gene frequency but form a frequency distribution. A typical result is shown in Figure 2 for parameter set 5. The abscissa of each histogram is marked off in gene frequency, p, of the + allele. Since the total number of individuals is eight, there are only 16 genes in the population at the t locus so that p is always a multiple of 1/16. The ordinate of each histogram shows the proportion of all populations (runs) having the given value of p. Results are plotted for generations 10, 20, 30, . . . 200. The trend in the observed frequencies (black bars) is obvious. As the number of generations increases, the proportion of populations reaching fixation of the + allele ($p = 1.0$) increases so that by generation 200, 84 percent of all populations are fixed. In the unfixed populations the most frequent class is $p = .50$ and the distribution is J-shaped. This J-shaped distribution is fairly stable in form over the entire set of 200 generations, the only change being a gradual decrease in the total frequency of unfixed populations. This stability is shown by the clear bars which represent the distribution of unfixed classes readjusted to give a total frequency of unity. That is, the clear bars show the proportion of *as yet unfixed* populations which fall in each class. The existence of such a stable distribution of unfixed classes is in accordance with the prediction of WRIGHT (1937).

Rates of fixation

The proportion of populations fixed for the normal allele in successive generations is given in Tables 4 and 5. Table 4 shows the proportion of previously unfixed populations which become newly fixed in each ten-generation period.

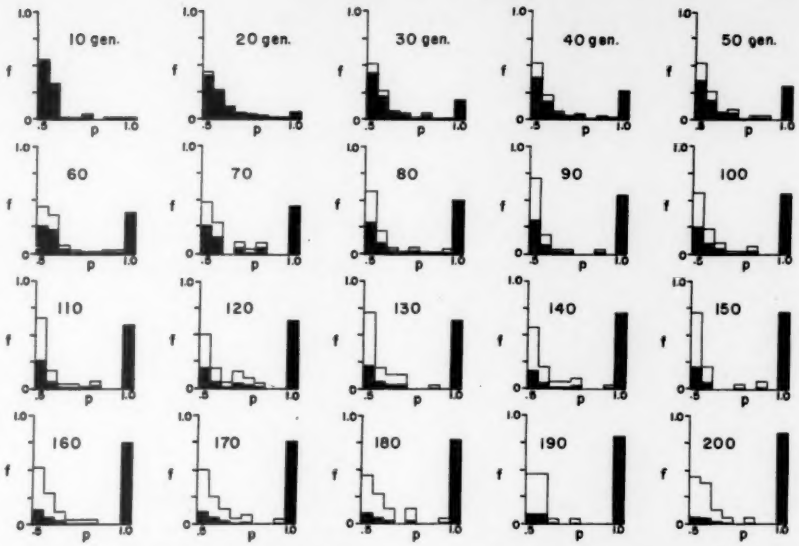

FIGURE 2.—Frequency distributions of gene frequencies in successive generations. The ordinate shows the proportion of all populations (runs) having a given gene frequency. The abscissa shows the values of p, the frequency of the normal allele. Black bars are the distributions of all runs. Clear bars are the conditional distributions for unfixed classes. Based on the data from parameter set 5.

They are then the *fixation rates* per ten generations. In Table 5 the cumulative proportions of fixed populations are given, and this latter information is plotted in Figure 3.

A number of interesting comparisons can be made by holding all parameters constant except one. The effect of a change in population size can be seen by comparing set 3 with set 5, and set 4 with set 10. Sets 3 and 5 both have a segregation ratio of .95 but set 3 with four males and four females has a higher effective population size ($N = 8$) than does set 5 ($N = 6$). The effective population size is calculated from the relation

$$N = \frac{4 N\delta \, N♀}{N\delta + N♀}$$

Similarly, sets 4 and 10 both have $m = .90$ but again have population sizes 8 and 6 respectively. As shown by the means at the bottom of Table 4, the fixation rates of the smaller populations are between one and a half and two times as large as for the larger populations. These mean fixation rates are roughly the equilibrium rates since generations 10 and 20 are not included in their calculation. The result of the difference in fixation rates between large and small populations is shown in Table 5 and Figure 3 as a difference in the proportion of populations fixed after n generations. In the case of set 10, for example, all populations are fixed by

TABLE 4

Fixation rates per ten generations

Generation	Set						
	3	4	5	6	8	9	10
10	.000	.040	.020	.218	.018	.207	.054
20	.000	.083	.051	.140	.036	.065	.189
30	.060	.113	.117	.125	.075	.070	.209
40	.064	.128	.108	.086	.020	.000	.294
50	.023	.059	.054	.125	.042	.075	.291
60	.070	.031	.101	.107	.022	.027	.059
70	.000	.129	.097	.060	.022	.083	.313
80	.075	.074	.107	.064	.045	.000	.181
90	.000	.120	.100	.068	.000	.000	.333
100	.054	.136	.022	.073	.071	.061	.000
110	.029	.000	.067	. . .	.026	. . .	.000
120	.059	.034	.095	. . .	.000	. . .	.333
130	.031	.111	.000	. . .	.000	. . .	.500
140	.064	.250	.184	. . .	.000	. . .	.000
150	.069	.000	.065	. . .	.026	. . .	.000
160	.037	.167	.103	. . .	.054	. . .	.500
170	.077	.000	.038	. . .	.029	. . .	.000
180	.083	.000	.080	. . .	.000	. . .	1.000
190	.091	.100	.130	. . .	.058	. . .	. . .
200	.250	.222	.150	. . .	.031	. . .	. . .
Weighted mean	.055	.103	.090	.093	.030	.041	.234

TABLE 5

Cumulative fixation rates. Proportion of all runs of each set which are fixed by a given generation

Generation	Set						
	3	4	5	6	8	9	10
10	.00	.04	.02	.22	.02	.21	.05
20	.00	.12	.07	.33	.05	.26	.23
30	.06	.22	.18	.41	.12	.31	.39
40	.12	.32	.27	.45	.14	.31	.57
50	.14	.36	.31	.53	.18	.36	.70
60	.20	.38	.38	.58	.20	.38	.71
70	.20	.46	.44	.61	.21	.43	.80
80	.26	.50	.50	.63	.25	.43	.84
90	.26	.56	.54	.66	.25	.43	.89
100	.30	.62	.55	.68	.30	.47	.89
110	.32	.62	.58	. .	.32	. .	.89
120	.36	.64	.62	. .	.32	. .	.93
130	.38	.68	.62	. .	.32	. .	.96
140	.42	.76	.69	. .	.32	. .	.96
150	.46	.76	.71	. .	.34	. .	.96
160	.48	.80	.74	. .	.37	. .	.98
170	.52	.80	.75	. .	.39	. .	.98
180	.56	.80	.77	. .	.39	. .	1.00
190	.60	.82	.80	. .	.43	. .	1.00
200	.68	.86	.83	. .	.45	. .	1.00

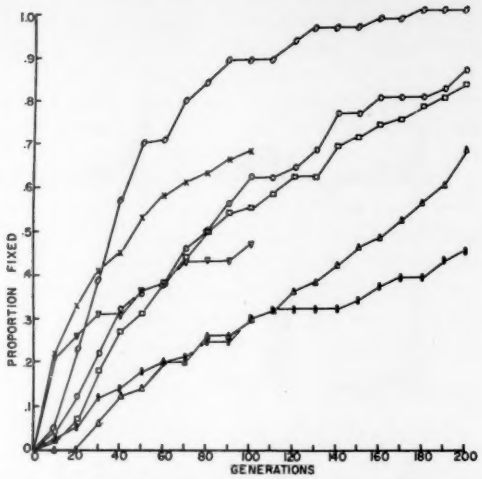

FIGURE 3.—Proportions of populations fixed for the normal allele in successive generations. Triangles: set 3; circles: set 4; squares: set 5; crosses: set 6; vertical bars: set 8; inverted triangles: set 9; oblique circles: set 10.

generation 180, while only 80 percent of the populations in set 4 are fixed at that time.

The effect of changes in segregation ratio can be estimated by comparing set 3 with set 4, and set 5 with set 8 and with set 10. In each case the lower the segregation ratio the greater the fixation rate. Sets 10, 5 and 8 have segregation ratios respectively of .90, .95 and .98. Their mean fixation rates are .234, .090 and .030. One way of comparing fixation rates is from the "half-life" of each set, that is, the number of generations required for 50 percent of the populations to be fixed. For sets 10, 5 and 8, these half-lives are approximately 36, 80 and 220 generations, the last figure coming from an extrapolation.

Finally, the role of different initial compositions can be studied. Sets 5 and 6 are identical except that each population in set 6 began with all wild type individuals with the exception of a single heterozygous male. Table 4 shows that the *mean equilibrium* fixation rates are virtually identical for these two sets (.090 and .093 respectively). The very large difference in total fixation between the sets as given in Table 5 and Figure 4 is entirely due to the high fixation rate in the first few generations. If the single mutant allele from the heterozygous male succeeds in "infecting" the population, there will no longer be any effect of its originally low frequency. The same phenomenon can be seen in the comparison between sets 8 and 9. The probability of a successful infection of a pure normal population with a t allele is fairly high in both cases. For set 6 ($m = .95$) the introduced t allele lasts more than 20 generations in 67 percent of the trials. The comparable figure for set 9 ($m = .98$) is 74 percent. As is to be expected, the higher the segregation ratio the greater the probability of successful infection.

In one set, 7, ($m = .95$) which is not shown in any detail, a single heterozygous

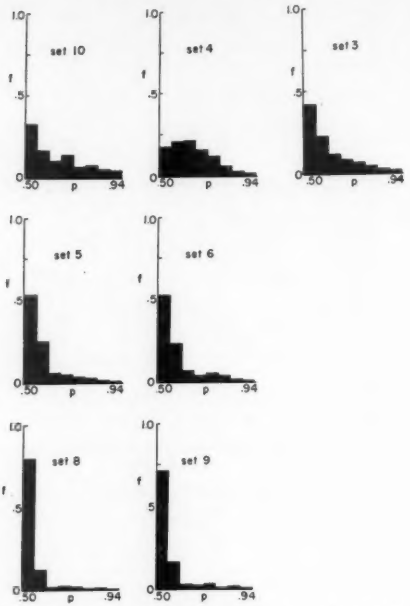

FIGURE 4.—Stable distributions of unfixed populations for various parameter sets.

female was introduced into an otherwise normal population. In 30 runs, only two populations remained unfixed for more than 20 generations (31 and 98 generations to fixation), two were fixed between the tenth and twentieth generations, and the remaining 26 populations were fixed before the seventh generation. Thus, the lack of an abnormal segregation ratio in the female makes it extremely unlikely that a *t* allele brought in by a female will survive. The difference between the case of migrant females and migrant males lies essentially in the difference between the initial frequencies of the *t* allele in the gametic pool. Clearly, it is not the initial adult composition that matters but the proportions of *T* and *t* gametes which they produce.

The probability of successful introduction of a mutant allele into an otherwise normal population is an important consideration in the maintenance of the polymorphism, and we shall return to this point in discussing the role of breeding structure and migration.

The stable distribution of gene frequencies

In addition to comparing fixation rates from one parameter set to another, we may also examine the effects of parameter changes on the distribution of unfixed populations. Figure 4 shows the stable distributions of sets 3, 4, 5, 6, 8, 9 and 10. These are constructed by pooling the distributions of generations 30–200 (30–100

for sets 6 and 8) to give an average distribution of unfixed classes. The first two periods are not used so that the initial population composition will not affect the results.

The effect of population size is shown by comparison of the distributions for set 3 with set 5, and set 4 with set 10. In both cases the smaller populations show a higher frequency in the extreme left-hand class, $p = .50$, and consequently lower frequencies in the other classes. Table 6 contains the probabilities associated with χ^2 tests for the differences between the distributions of different parameter sets. As these probabilities show, the effects of changing the population size are highly significant. The piling up of populations in the left-hand class is a result of the asymmetry of natural selection. A smaller population size causes greater genetic drift both toward lower and higher frequencies of the + allele with a consequent depletion of intermediate classes. Since t/t homozygotes are lethal, however, the frequency of the + allele can never fall below 0.50. The populations that would ordinarily go to fixation at $p = 0$ are then piled up at the barrier imposed by selection at $p = .50$.

Another view of the same effect can be obtained from the means and variances of the distributions as listed in Table 7. The results of t tests for differences between these means are given in Table 6. In discussing the large populations it was noted that the mean values of p were significantly lower than the theoretical

TABLE 6

Probabilities resulting from tests for the difference between parameter sets that differ in one parameter

Set	3	4	5	6	8	9	10
3		<.001	<.001	...	...	...	...
4	<.001		...	...	...	...	.001
5	<.001	...		.84	<.001	...	<.001
6	...	...	.60		...	...	...
8	...	...	<.001	...		.27	<.001
9	...	...	...	...	.12		...
10	...	.80	<.001	...	<.001	...	

Above the diagonal: χ^2 tests for the difference between the stable distributions of Figure 4.
Below the diagonal: t tests for the difference between means of Table 7.

TABLE 7

Means and variances of the stable distributions of unfixed classes of Figure 4

Set	$\bar{p}$	$\bar{q}$	σ_p	σ_p	$\sigma_{\bar{p}}$	95 percent conf. int. for p	$P_{theor.}$
3	.5935	.4065	.0130	.1139	.0047	.5843–.6027	.6147
4	63.88	.3612	.0117	.1079	.0058	.6275–.6501	.6667
5	.5656	.4344	.0101	.1003	.0036	.5586–.5726	.6147
6	.5692	.4308	.0109	.1043	.0052	.5591–.5793	.6147
8	.5290	.4710	.0056	.0746	.0028	.5235–.5345	.5714
9	.5380	.4620	.0067	.0818	.0049	.5285–.5475	.5714
10	.6356	.3644	.0186	.1363	.0115	.6131–.6581	.6667

values derived from BRUCK's equation. This is even more true of the small populations, and those with effective size 6 deviate more from the theoretical values than those with effective size 8, although the difference between sets 4 and 10 is clearly not significant. This lowering of the mean of unfixed classes is a result of the greater genetic drift with a piling up of populations at the lower limit of $p = .50$.

The consequence of a change in segregation ratio is reflected in the difference among sets 10, 5 and 8. As Figure 4 shows, an increase in m from .90 to .95 to .98 causes a piling up of the distribution at the left end. These differences are significant (Table 6) and are perfectly paralleled by a drop in the mean value of p from .6356 to .5656 to .5290 respectively. The concentration of the unfixed classes around $p = .50$ for high segregation ratios is also in accord with the slower rate of fixation observed for these parameter sets.

Finally, the lack of any detectable effect of initial composition on the equilibrium rate of fixation is paralleled by a lack of difference in the stable distributions of unfixed populations. The distribution for set 5 is virtually identical with that for set 6, and an almost equal degree of similarity exists between the distributions for sets 8 and 9. The χ^2 tests for differences between 5 and 6 and between 8 and 9 are nonsignificant as contrasted with the other comparisons in Table 6. The similarity between the distributions for sets 5 and 6 and for sets 8 and 9 is also shown by their means in Table 7.

Comparisons with populations in nature

The observations on the geographic distribution of t alleles were obtained before the models were derived and were primarily designed to detect and accumulate for genetic study a variety of t alleles from different sources. Gene frequencies were thus obtained only as by-products of this general sampling process, and in nearly all cases are based on very small samples. While they are adequate to give some clues to the state of this locus in wild populations, they cannot serve as strict tests of the applicability of the models to conditions in nature.

Mice were obtained from traps set in 31 localities distributed over the United States with one site sampled in Nova Scotia; and in addition samples were obtained from three enclosed populations descended from wild mice captured in three additional localities. The methods of trapping varied. Usually one or a few traps were set to test whether Mus was present. At first this was done in buildings; later eight feral populations were sampled. Since the object was mainly to capture mice from the locality, it is quite possible that mice caught at different times in the locality came from different breeding units or families, and there is some suggestion in the testing records that this occurred. It is evident therefore that mice from different trapping stations in the same locality may or may not represent samples from the same breeding unit. Although the mice in the localities of capture have been referred to as "populations," this has only a general geographic, not a strict biological meaning.

All animals were tested individually by matings to Brachys ($T/+$) at Nevis

Biological Station. At least one heterozygote ($+/t^w$) was found in the sample from each of 14 localities and from each of three enclosed populations. Of 66 mice tested from 14 localities, 23 were $+/t^w$. Sample sizes per locality ranged from one to ten. It is obvious that because of small and variable sample sizes and the method of trapping, no reliable gene frequency can be based on the above. It does show, however, that heterozygotes for these alleles are rather common (minimum frequency about 35 percent) in the populations in which they occur.

Better estimates can be obtained from extensive tests of enclosed populations bred in captivity. One of these has been maintained for 13 years at the Rockefeller Institute for Medical Research in New York, having originated from a few wild mice captures in New York City and Philadelphia in 1946. The breeding colony is kept at 125–250 individuals in the winter, reduced to about a dozen pairs each summer, so considerable inbreeding has occurred. Four samples from this population kindly supplied by DR. HOWARD SCHNEIDER, have been tested (Jan. '52, June '52, June '55, June '59). Heterozygote frequencies have remained at 50–60 percent. Up to now, of 197 animals tested 110 (or 56 percent) have been $+/t^w$.

One population in Rumford, Virginia, which was sampled by A. B. BEASLEY yielded in 1955 one $+/t^w$ out of eight tested. In the spring of 1959 an attempt was made by JOHN BEASLEY to trap all the animals in this barn. Of 41 animals captured 14 have completed tests. Eight of these (57 percent) are $+/t^w$. A similar attempt was made to capture all mice in a feed room of an isolated barn near Storrs, Connecticut. Of seven which bred in captivity, five were $+/t^w$. These experiences suggest that where *t* alleles occur, heterozygotes reach frequencies of 50–60 percent; the scattered small samples yield estimates below this value.

Testing of successive samples from Clinton, Montana, supplied by MISS VIRGINIA VINCENT suggest a possible reason for such discrepancies. A sample of 11 taken from this station (in a small barn) in September 1957 all proved to be $+/+$; a sample of three taken in October 1958 all proved to be $+/t^w$. Some of the latter may have come from the nearby house. The combined estimate is 3/14, but this may include family groups with high and with low frequencies.

Samples from 17 localities failed to yield a $+/t^w$ animal. The sample sizes tested were in general small, in 13 cases five or less. From only one locality (Great Gull Island in Long Island Sound) were sufficient mice tested (40) to give a high probability that *t* alleles were not present.

The general picture that emerges from this survey is that local groups of Mus free from *t* alleles are probably rare in the continental area. The only one established as *t* free is on a well isolated coastal island. Where *t* alleles occur they seldom or never reach the frequencies predicted by the deterministic model based on the average male segregation ratios actually found.

An effect of applying the stochastic model to small breeding groups is to suggest a reason for the failure of the deterministic model to fit the actual results. The equilibrium predicted by the latter model is not attained because of the chance loss of such alleles in small breeding groups. The general trend in the small groups is clearly toward fixation of wild type alleles. This process is, to be sure, retarded

by very high values of the segregation ratio, but the direction is clear; an appreciable portion become fixed with the passage of time.

This process would produce a dichotomy amongst family groups in the same locality, some fixed, others unfixed, and the average gene frequencies for the locality would be determined largely by the proportions between such groups in the locality.

The sampling methods used heretofore are inadequate to test whether something like the above situation obtains in nature, but at least we know now what kind of observations must be made. Family and household structure must be known, and methods designed for the capture of households or extended families.

In the few cases in which collections of families have been studied (Rockefeller and Virginia samples) the average gene frequencies are not far from those predicted on the stochastic model and far below those predicted on the deterministic model. This cannot be taken as validation of the model until other variations in parameters have been shown to correspond to those assumed in the model.

A new and useful view of the methods by which *t* alleles may spread to new populations is also given by the analog calculations. It appears that alleles must travel in males in order to have a good chance of persisting in the newly "infected" population. This gives new impetus to studies of relative mobilities of the two sexes as factors in gene dispersal. This may have special importance in this case, since an implication of the stochastic model is that *t* lethals are at best "genes of passage" in small breeding groups because of the probability of chance loss. Those which are found, therefore, may have been acquired rather recently from migrants. This is more probable than acquisition by mutation since, as yet, we know of no instance of mutation of the wild type allele to *t*.

A final point which emerges from the stochastic model concerns selection among the various *t* alleles. Table 1 shows that all the *t* alleles found in natural populations exhibit very high segregation ratio abnormalities. This is not true, however, of all "mutant" alleles at this locus. In addition to the alleles of wild origin there are 19 alleles at this locus which have arisen in the laboratory. These arose from balanced lethal lines of the type T/t^n, in the form of single normal-tailed individuals which later proved to be t^n/t^z. When the segregation ratios of the newly arisen laboratory "mutants" are compared with alleles from wild populations, a radical difference, shown in Table 8, is found. Newly arisen alleles have segregation ratios ranging from very high to very low with a distinct mode at .50. Clearly the wild alleles are a selected sample of all the possible "mutations"

TABLE 8

Distribution of male segregation ratios for t *alleles extracted from wild populations and for* t *alleles arisen in the laboratory by mutation from other* t *alleles*

	.99 .90	.89 .80	.79 .70	.69 .60	.59 .55	* .50	.49 .45	.44 .40	.39 .30	.29 .20	*n*
Wild alleles	15	1	0	0	0	0	0	0	0	0	16
Newly arisen alleles	3	1	0	0	2	8	2	1	2	0	19

* Ratios within $\pm$ 2 σ of .5, i.e., normal ratios.

at this locus, selected for high segregation ratio in favor of the mutant. The deterministic model would not predict such selection since any mutant allele with a segregation ratio above .50 would be maintained in a stable equilibrium. The stochastic model, however, does predict the selection of mutants with higher segregation ratios. The Monte Carlo results showed the rate of loss of *t* alleles due to random drift to be very sensitive to the value of *m*. Alleles with relatively small segregation advantages would be lost quite rapidly with the result that the only alleles still seen in natural populations are those with high values of *m*. As time goes on this concentration of alleles at the high end of the range should become even sharper. These considerations once more emphasize that the polymorphism for *t* alleles is a transient one and very probably subject to violent oscillations. New alleles arise occasionally from old ones by a process which is not understood but which may involve recombination. Such new alleles as have high segregation ratios have a high probability of spreading in the population and of "infecting" other populations by the migration of heterozygous males. The alleles then spread in the species but are constantly being lost by drift and revived by migration. Eventually they must be lost entirely only to be replaced by yet another rare mutant of high segregation advantage.

SUMMARY

Wild populations of *Mus musculus* are polymorphic for mutant alleles at the *t* locus. When homozygous, these alleles are usually unconditional prenatal lethals, but a few are viable and male-sterile. Heterozygous males have an average of 95 percent *t*-bearing sperm in their effective sperm pool. This abnormal segregation ratio opposes the loss of the *t* alleles due to their lethal effect. Deterministic models for the evaluation of this polymorphic system predict (1) that any allele with a segregation ratio above .50 should be maintained in a stable equilibrium, and (2) that alleles with segregation ratios of the order of 95 percent should be in such high frequency as to make between 60 and 95 percent of the population heterozygous. Neither of these predictions is in accord with the observations from nature since: (1) only alleles with very high segregation ratio are found in contrast to the range known in laboratory mutants and (2) populations contain about 35 percent to 50 percent heterozygotes.

A stochastic model has been constructed, and results of this model were obtained by Monte Carlo procedures on a digital computer. These results show that for populations composed of small family groups there will be a loss of the *t* allele due to random genetic drift. The rate of loss is sensitive to differences in segregation ratio so that only alleles with a high ratio will remain in the population for appreciable periods. A geographical population will be composed mainly of family groups fixed for the wild type allele and of family groups with a high frequency of the mutant *t* allele. A normal population is easily "infected" with a mutant allele if this allele is carried into the population by a heterozygous male. These results are all in good accord with observations from nature insofar as the sampling methods thus far used permit a comparison.

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GENETICS OF NATURAL POPULATIONS. XXVIII. THE MAGNITUDE OF THE GENETIC LOAD IN POPULATIONS OF DROSOPHILA PSEUDOOBSCURA

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POPULATIONS of species that reproduce sexually and by cross-fertilization usually carry some genetic variants which, either when homozygous or both in heterozygous and in homozygous condition, decrease the fitness of their carriers in at least some environments. The sum total of genetic variants of this kind, present in the gene pool of a population, constitutes its genetic load. Exploration and analysis of genetic loads in populations of the same and of different species living in the same or in different environments is one of the most urgent tasks of modern population genetics. Of late, this topic has acquired added interest, especially in connection with the problem of genetic damage that may be caused by the exposure of human and other populations to high-energy radiations. The genetic damage consists in making the genetic load of a population heavier than it was before the damage was inflicted.

Quantitative studies on genetic loads generally require special genetic tools, chromosomes with suitable "marker" genes, inversions, crossover suppressors, etc. Genetic tools of this sort are at present available only in a few species of Drosophila. It is not beyond the realm of what is possible to contrive such tools also in corn and perhaps in some other organisms, but this has not, in fact, been accomplished to date. Other and much less direct techniques will have to be used in studies on the genetic loads of human populations, and it is perhaps not unduly optimistic to hope that studies on Drosophila may be useful in pointing the way.

The pioneering studies on the genetic loads in populations of *D. melanogaster* were initiated by CHETVERIKOV (1927), DUBININ and his collaborators (1934 and later works), IVES (1945 and later), and others. In *D. pseudoobscura*, the species with which the present work is concerned, the genetic loads were investigated by STURTEVANT (1937); DOBZHANSKY and QUEAL (1938); DOBZHANSKY (1939); DOBZHANSKY, HOLZ and SPASSKY (1942); DOBZHANSKY and SPASSKY (1944, 1953, 1954); and DOBZHANSKY, PAVLOVSKY, SPASSKY, and SPASSKY (1955). The available data remain, nevertheless, fragmentary and unsatisfactory in several ways.

(1) All the experiments were made with the aid of a technique which involved comparisons of the viability of individuals homozygous for a given wild chromo-

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some with individuals heterozygous for the same chromosome. This is a method for the detection of recessive genetic variants. A superior method was evolved by WALLACE (1956); it makes all the test cultures contain a class of flies which do not carry the chromosome being tested at all. This class serves as a common denominator, and its presence permits detection of dominant effects of the tested chromosomes.

(2) The old experiments have shown that the viability for homozygotes for many wild chromosomes is extremely sensitive to environmental variations. We do not, however, know how the frequencies of the different viability classes (lethals, semilethals, subvital, and supervital chromosomes) would compare if tested under more stringent and at more optimal environmental conditions.

(3) Some data have suggested that, at least in Drosophila, individuals homozygous for wild chromosomes are less homeostatic (i.e., more sensitive to environmental changes) than individuals whose two chromosomes of a pair are derived from not closely related parents. This has been questioned by some writers, and misinterpreted and misconstrued by others; further data are obviously needed.

(4) How a chromosome behaves in homozygotes may not matter greatly in outbred populations in which the probability of any one chromosome being present in duplicate in any individual is slight. More important is what a chromosome does in heterozygotes (see above, point 1). It is desirable to know whether individuals carrying different combinations of chromosomes are phenotypically uniform or heterogeneous, and if heterogeneous, whether the heterogeneity is correlated with the effects of the component chromosomes in homozygotes. The experiments described in this, and on the following articles, were designed to test these points.

MATERIAL AND EXPERIMENTAL PROCEDURE

A sample of the natural population of *D. pseudoobscura* from the vicinity of Austin, Texas, was collected in March, 1957 by MR. T. G. GREGG. The population at Mather, California, was sampled in June of the same year by one of us (TH. DOBZHANSKY). The analysis of the samples was carried out in the laboratory in New York.

For analysis of the second chromosomes, wild males, or sons of wild females, were outcrossed to females having the dominant gene Bare (*Ba*, short bristles) and the recessive glass (*gl*, disarranged eye facets) in one of the second chromosomes, and the dominant Delta (Δ, expanded wing veins) in the other second chromosome. The *Ba gl* chromosome had also an inversion which suppressed most of the crossing over. In the F_1, a single *Ba gl*/+ male was taken, and crossed to *Ba gl*/Δ females from the stock culture. In the F_2, groups of eight *Ba gl*/+ females and like numbers of Δ/+ males were selected and transferred to fresh culture bottles six times at one- to two-day intervals, at room temperatures. Half of the resulting bottles with eggs deposited in them were placed in a constant temperature room at 25°C, and the other bottles in a similar room at 15°-16°C. When the progenies hatched, they were counted four times at three-day intervals

in the bottles at 25°C and at five-day intervals at 15°C. The progenies should contain four types of flies, in equal numbers, as follow: wild (+/+), Bare (*Ba gl*/+), Delta (Δ/+), Bare-Delta (*Ba gl*/Δ).

The wild type flies carry in duplicate (are homozygous for) the wild second chromosome under test; the Bare and Delta flies carry the same chromosome in heterozygous condition; the Bare-Delta flies do not carry this chromosome at all.

For the analysis of the third chromosomes, an analogous series of crosses was made. Wild males, or sons of wild females, were crossed to females having the dominant Lobe (*L*, eye shape), the recessive orange (*or*, eye color), and an inversion (Santa Cruz) in one third chromosome, and the dominant Blade (*Bl*, wing shape), Scute (*Sc*, some bristles absent), and the recessive purple (*pr*, eye color) in the other third chromosome. A single F_1 *Bl Sc*/+ male from each cross was mated to *L or*/*Bl Sc pr* females from the stock culture; in the next generation groups of eight *L or*/+ females and *Bl Sc pr*/+ males were taken and allowed to oviposit consecutively in six culture bottles. As with the second chromosomes, half of the bottles were then kept at 25° and half at 15°C. The progenies contained four classes of offspring, namely: wild (+/+). Blade-Scute (*Bl Sc pr*/+), Lobe (*L or*/+), Blade-Scute-Lobe (*Bl Sc pr*/*L or*).

The wild type flies are homozygous for the wild third chromosomes which are being tested; the Blade-Scute and the Lobe flies carry these wild chromosomes in single dose; the Blade-Scute-Lobe flies are free of these chromosomes. These four classes should be equally frequent among the zygotes formed, but among the adult flies their frequencies are quite variable because of the variations in the viability. The homozygous class, +/+, varies from complete lethality to normality and to supervitality.

RESULTS AND DISCUSSION

The normal viability standards: The average viability in a given environment of flies which carry the two chromosomes of a pair taken at random from a given population is regarded as "normal" for that population. Since the chromosomes derived from not closely related parents will usually differ somewhat in their gene contents, the "normal" flies will be mostly heterozygous. They will be referred to below as "heterozygotes", to distinguish them from the "homozygotes" which carry certain chromosomes in duplicate. Operationally, the standards of normal viability can be arrived at by means of rather simple control experiments.

For the control of second chromosomes, *Ba gl*/+ females and Δ/+ males were taken from the progenies of two *different* wild parents, and intercrossed. As in the main experiments, six bottles were obtained with eggs deposited by the same group of eight females and eight males; three bottles were allowed to develop at 25°C and the three others at 15°C. The +/+ class obtained in the progeny carries, then, the two + chromosomes derived from different ancestors. The average viability of the flies of this class is normal by definition. The control experiments for the third chromosomes involved, analogously, crosses of eight *L or*/+

females × eight *Bl Sc pr*/+ males from *different* cultures. Again six cultures were raised for tests of each pair of chromosomes, three of them at 25° and three at 15°C. The counts in the control cultures were made exactly as in the cultures of the main experiment dealing with the homozygotes.

Table 1 reports a summary of the results separately for the second and the third chromosomes, for the two temperatures, and for the chromosomes of Texas and of California origins. The frequencies of the +/+ class, the average viability of which is normal by definition, may be calculated in two ways. First, the frequencies may be expressed in percentages of the total numbers of all the flies counted in the test cultures. Secondly, we may calculate the ratios of the numbers of +/+ flies to Bare-Delta (or respectively, Blade-Scute-Lobe) flies in the same cultures. Table 1 reports both the percentages and the ratios.

Table 1 shows that the frequency of the +/+ is invariably higher in the experiments involving chromosomes of California origin than in those with chromosomes of Texas origin. The crosses are such that the Y chromosome comes from the wild population, the X from the laboratory stock, and on the average ¾ of the gene content of the uncontrolled autosome comes from the laboratory stock. The higher frequency of +/+ when the chromosomes are of California origin may be due either to a direct effect of these chromosomes on this background under laboratory conditions or to a different effect on viability of the dominant mutant genes used as markers in the experiments (i.e., *Ba*, Δ, *Bl*, *Sc*, and *L*) when the wild component of the genetic background is of California rather than Texas origin.

The normal viability is defined as the *average* viability of the +/+ class in the control experiments. Different pairs of wild chromosomes yield, however, somewhat different percentages of +/+ flies, as reported in Figure 2. It will be shown below that these differences are greater than could be accounted for by sampling errors and environmental fluctuations.

Viability of the homozygotes: The frequencies of the +/+ flies in the cultures in which this class of flies was homozygous for different wild chromosomes are summarized in Figure 1, which shows the numbers of chromosomes yielding various percentages of +/+ flies among the flies counted in the test cultures.

TABLE 1

The "normal" viability standards. Mean percentages and mean ratios of the heterozygous +/+ class in the control experiments

Chromosome	t°C	Locality	Percentages Mean	σ	Ratios Mean	σ	Chromosome combinations
Second	25°	Texas	26.03±0.31	2.20	1.103±0.023	0.160	49
Second	25°	Calif.	27.12±0.49	2.85	1.179±0.031	0.189	34
Second	15°	Texas	26.31±0.36	2.56	1.108±0.027	0.196	52
Second	15°	Calif.	27.14±0.46	2.66	1.234±0.033	0.191	33
Third	25°	Texas	24.68±0.31	2.30	1.010±0.021	0.156	56
Third	25°	Calif.	28.12±0.45	2.29	1.288±0.039	0.199	26
Third	15°	Texas	24.27±0.40	2.87	1.006±0.028	0.205	52
Third	15°	Calif.	26.31±0.37	1.88	1.088±0.029	0.146	26

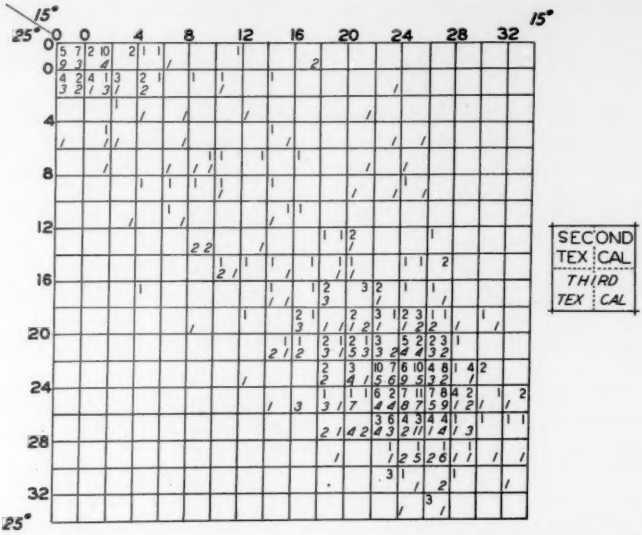

FIGURE 1.—Viability of the homozygotes for different chromosomes, as measured by the percentages of the wild type class observed in the cultures raised at 25°C (ordinates) and at 15°C (abscissae). The figures for the second and the third chromosomes of Texas and of California origins are placed as shown in the diagram on the right.

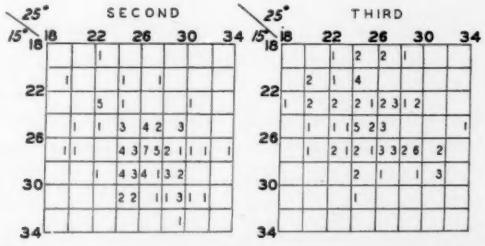

FIGURE 2.—Viability of the heterozygotes for different combinations of chromosomes, as measured by the percentages of the wild type class observed in the cultures raised to 25°C (abscissae) and at 15°C (ordinates). The figures on the left in the squares are for the Texas chromosomes, and on the right for California chromosomes.

These frequencies could be also expressed as ratios of +/+ to Bare-Delta, or to Blade-Scute-Lobe, flies (as was done in Table 1); the two representations give the same picture, but the ratios are more variable. Figure 1 is constructed like a correlation table; the entries in each cell show the numbers of the chromosomes which yielded certain percentages of +/+ homozygotes in test cultures at the two temperatures, 25° and 15°C. In order to represent the data for the second and

for the third chromosomes of Texas and of California origins in the same figure, the numbers in each cell are placed as shown in the scheme on the right. The mean viabilities of the homozygotes for the chromosomes studied (i.e., the mean percentages of the +/+ in the cultures) are shown in Table 2; these figures may be compared with the mean viabilities of the heterozygotes shown in Table 1.

Comparison of Tables 1 and 2 shows that the mean viability of the homozygotes is consistently lower than that of the heterozygotes. If the populations studied suddenly became homozygous for their second chromosomes, the resulting loss of fitness, expressed in percentages of the normal viability would be:

Texas, at 25°C—24.8 percent	California, at 25°C—26.2 percent
Texas, at 15°C—31.8 percent	California, at 15°C—31.9 percent

Homozygosis for third chromosomes would result in a somewhat smaller loss of viability, namely:

Texas, at 25°C—22.9 percent	California, at 25°C—26.5 percent
Texas, at 15°C—20.9 percent	California, at 15°C—23.9 percent

Since the second chromosome is cytologically longer, and presumably carries more genes than the third, this difference between the chromosomes is not unexpected. It should be noted that the loss of fitness in the relatively more stringent environment at 25°C is not much, if any, greater than in the more favorable environment at 15°C. Table 2 also shows that the values for Texas are not significantly, but consistently, lower than for California chromosomes. Since the same has been observed in the control experiment (see above), the inference that the marking genes used interact less favorably with the California than with the Texas genotype is strengthened.

Chromosomes which yield no +/+ flies in the test cultures (the zero class in Figure 1) are lethal when homozygous. Semilethal chromosomes reduce the viability of the homozygotes to less than one half of normal. The normal survival rates are shown by the control figures in Table 1. The percentages of lethal and semilethal chromosomes combined turn out to be as follows:

	Second chromosome	Third chromosome
Texas, 25°C	25.6 ± 3.5	20.3 ± 3.2
Texas, 15°C	22.1 ± 3.3	20.4 ± 3.3
California, 25°C	22.7 ± 3.6	22.9 ± 3.6
California, 15°C	21.5 ± 3.6	16.4 ± 3.1

There is no significant difference in the incidence of lethal and semilethal chromosomes between the Texas and California populations studied. Combining the data for the two localities, we have:

	Percentage	Chromosomes examined
Second chromosomes, 25°C	24.3 ± 2.5	288
Second chromosomes, 15°C	21.8 ± 2.5	284
Third chromosomes, 25°C	21.5 ± 2.4	293
Third chromosomes, 15°C	18.5 ± 2.3	292

TABLE 2

The viability of homozygotes for the chromosomes studied, expressed in mean percentages of the +/+ class in the cultures

Chromosome	t°C	Locality	Mean	σ	Chromosomes studied
Second	25°	Texas	19.58±0.73	9.06	156
Second	25°	Calif.	20.03±0.88	10.08	132
Second	15°	Texas	17.95±0.74	9.18	154
Second	15°	Calif.	18.46±0.88	10.01	130
Third	25°	Texas	19.00±0.68	8.47	153
Third	25°	Calif.	20.66±0.71	8.44	140
Third	15°	Texas	19.19±0.72	8.82	152
Third	15°	Calif.	20.03±0.81	9.60	140

The incidence of lethals is slightly, but again not significantly higher at 25° than at 15°C. It is interesting to compare the incidence in the present material with that observed in older experiments, all of which were conducted at 25°C. The relevant figures are as follows:

	Second chromosomes	Third chromosomes
Mather, California, 1957	22.7 ± 3.6	22.9 ± 3.6
Mather, California, 1951	33.0 ± 4.5	25.0 ± 4.0
San Jacinto, Calif., 1940–41	21.3 ± 1.8	13.9 ± 1.0
Death Valley, Calif., 1937	.....	17.0 ± 1.3
Austin, Texas 1957	25.6 ± 3.5	20.3 ± 3.2
Austin, Texas 1953	31.5 ± 3.5	.....
Mexico and Guatemala, 1938	.....	30.0 ± 4.2

The genetic loads are clearly greater in some geographic populations of the species than in others. It may also be that the load does not remain constant with time in the same population. Thus, the incidence of lethal second chromosomes at Mather, California, decreased by 10.3 ± 5.7 percent between 1951 and 1957. This does not reach the conventional level of statistical significance. However, IVES (1954) did observe changes with time in the genetic load of the Massachusetts population of *D. melanogaster*.

Subvital and normal chromosomes: Disregarding the chromosomes which are lethal or semilethal in homozygotes, we are left with an array of quasi-normal chromosomes. The mean viabilities of the homozygotes for quasi-normal chromosomes are shown in Table 3. Comparing the data in this table with those in Table 1 and 2, it can be seen that in every instance the mean viability of the quasi-normal homozygotes is intermediate between that of the heterozygotes and that of random homozygotes (i.e., including the lethals and semilethals). Some of the quasi-normal chromosomes are subvital when homozygous.

The conventional boundary between the subvital and semilethal chromosomes lies at 50 percent of normal viability. It is more difficult to delimit the subvital from the normal and the rare supervital chromosomes; this is because the via-

TABLE 3

The viability of quasi-normal homozygotes, expressed in mean percentages of the +/+ class in the cultures

Chromosome	t°C	Locality	Mean	σ	Chromosomes studied
Second	25°	Texas	23.04±0.34	3.61	110
Second	25°	Calif.	23.55±0.35	3.52	100
Second	15°	Texas	24.24±0.33	3.49	110
Second	15°	Calif.	25.14±0.31	3.12	100
Third	25°	Texas	23.44±0.34	3.66	115
Third	25°	Calif.	24.91±0.35	3.57	105
Third	15°	Texas	22.90±0.34	3.62	115
Third	15°	Calif.	24.41±0.30	3.03	105

bilities of the quasi-normal chromosomes form a bell-shaped distribution, the mean and the mode of which lie clearly below those for heterozygotes (cf. Tables 1 and 3). A method of estimation of the incidence of subvital chromosomes was evolved by WALLACE and MADDEN (1953; see also DOBZHANSKY and SPASSKY 1953, 1954). A brief statement will suffice here. We have defined the normal viability as the mean of the viabilities of random heterozygotes. However, the observed frequencies of the +/+ class in the cultures in which this class is heterozygous are variable. The observed, or total, variance (σ^2_{ges}) is due in part to sampling errors (σ^2_s), in part to environmental fluctuations (σ^2_e), and in part to genetic differences among the heterozygotes (σ^2_g). All these values are calculable from our data (see the papers referred to above for detail). Subvital chromosomes may, then, be defined as those yielding homozygotes with viability more than two standard deviations ($2\sigma_g$) below the normal mean, but higher than semilethal chromosomes (i.e., higher than 50 percent of the normal viability).

The estimates of the total variance, and of its components, for the homozygotes and the heterozygotes obtained in our experiments are summarized in Table 4.

TABLE 4

Observed variance (σ^2_{ges}), variance due to environment (σ^2_e), to sampling errors (σ^2_s), and the genetic variance (σ^2_g) among homozygotes for quasi-normal chromosomes and among heterozygotes

Chromosomes		t°C	Homozygotes				Heterozygotes			
			σ^2_{ges}	σ^2_e	σ^2_s	σ^2_g	σ^2_{ges}	σ^2_e	σ^2_s	σ^2_g
Second,	Texas,	25°	16.00	4.18	3.68	8.14	5.11	3.16	3.26	—1.31
Second,	Texas,	15°	15.58	1.99	3.00	10.59	6.30	2.06	3.27	0.97
Second,	Calif.,	25°	13.30	2.96	3.39	6.95	8.44	3.24	3.48	1.72
Second,	Calif.,	15°	11.25	2.51	3.28	5.46	7.23	3.52	3.59	0.12
Third,	Texas,	25°	11.73	4.07	4.14	3.52	5.44	2.01	3.90	—0.47
Third,	Texas,	15°	12.88	4.74	3.27	4.87	9.23	3.27	4.24	1.72
Third,	Calif.,	25°	15.45	4.32	3.42	7.71	5.43	0.61	3.52	1.30
Third,	Calif.,	15°	10.65	3.04	3.27	4.34	3.72	1.00	3.25	—0.53

It can be seen that the sampling variances (σ^2_s) are about the same for the homozygotes and the heterozygotes. This means only that the numbers of the flies per culture were nearly the same in homozygous and heterozygous cultures (see below). The environmental variances (σ^2_e) are higher on the average for the homozygotes, although the data contain three ostensible exceptions to this rule. This situation will also be considered in more detail below. What is important here is that the genetic variances (σ^2_g) are strikingly and consistently greater in homozygotes. In fact, some of the estimates of σ^2_g for heterozygotes are negative, which only means that the observed total variance (σ^2_{ges}) was in these cases smaller than could be accounted for by sampling errors and environmental fluctuations. Using, then, the WALLACE-MADDEN method, we arrive at the following estimates of the percentages of quasi-normal chromosomes which are subvital when homozygous:

		Second chromosomes	Third chromosomes
Texas	25°C	89.6 percent	81.1 percent
Texas	15°C	56.0 percent	29.5 percent
California	25°C	69.1 percent	68.0 percent
California	15°C	81.6 percent	86.7 percent

The only comparable estimates in the literature are those of DOBZHANSKY and SPASSKY (1953) for subvital chromosomes in the Mather, California, population in 1951. These estimates are 93.5 percent for the second and 41.3 percent for the third chromosomes. It is unfortunately difficult to assign any confidence ranges for these estimates. The experimental errors involved are undoubtedly considerable. Averaging up all the available figures, we obtain 78 percent for the second and 61 percent for the third chromosomes; these are probably the best estimates of the frequencies of subvital chromosomes in natural population of *Drosophila pseudoobscura* now obtainable. Estimates of the frequencies of supervital chromosomes would be even less reliable.

Modification of the viability by temperature change: As stated above in the description of the experimental procedure, we have examined the viability of the homozygotes for certain chromosomes and of the heterozygotes for certain combinations of chromosomes by raising three replicate test cultures at each of the two temperatures, 25° and 15°C. We shall now inquire as to what extent the viability is modifiable by environmental changes. The differences between the cultures kept at 25° and at 15°C may be referred to as "macroenvironmental" and those between the cultures kept at the same temperature as "microenvironmental". The data for the macroenvironmental variations are summarized in the form of correlation tables in Figure 1 for the homozygotes, and in Figure 2 for the heterozygotes.

With the homozygotes it is obvious, at a glance at Figure 1, that the performances of a chromosome at the two temperatures are strongly correlated; the chromosomes which are lethal at one temperature are mostly lethal also at the other; the chromosomes which are quasi normal at 25°C are mostly quasi normal at 15°C. However, some striking modifications of the viability record are observed.

Thus, 22 of the chromosomes which were lethal at 25°C (0 on the vertical axis) were semilethal at 15°C (0 to 14 percent on the horizontal axis), and two were even subvital (16 to 18 percent). Of the chromosomes which were lethal at 15°C, only one became definitely semilethal (4–6 percent), and 11 produced up to two percent of the +/+ class. Some of the chromosomes which were semilethal at 25°C became subvital or even normally viable at 15°C; transformation from semilethality at 15°C to subvitality at 25°C seems to be less frequent. No such sharp changes occur among heterozygotes (Figure 2). The viability is evidently most variable among those homozygotes which are lethal or semilethal at least at one of the temperatures used in our experiments.

It is less evident, and therefore important to prove, that there is a real diversity also among the quasi-normal chromosomes. The same problem arises with respect to the diversity of the heterozygous chromosome combinations. We have, therefore, computed the correlation coefficients from the data summarized in Figures 1 and 2. In making the calculations, we have chosen to consider only those homozygotes which yielded no less than 14 percent of +/+ flies in the tests at both temperatures. (In so doing, we have ignored a few chromosomes which behaved as quasi normals at one temperature but as extreme subvitals or semilethals at the other). All the heterozygous combinations which were tested at both temperatures were utilized. The correlation coefficients (r) obtained are as follows:

	Second chromosomes	Third chromosomes
Texas, homozygotes	$+0.46 \pm 0.08$	$+0.23 \pm 0.09$
California, homozygotes	$+0.27 \pm 0.09$	$+0.31 \pm 0.09$
Texas, heterozygotes	$+0.39 \pm 0.12$	$+0.16 \pm 0.14$
California, heterozygotes	$+0.32 \pm 0.16$	$+0.25 \pm 0.18$

The correlation coefficients are in every instance positive, although some of them do not attain the conventional level of statistical significance. We conclude that the homozygotes for different quasi-normal chromosomes as well as the "normal" heterozygotes, are not uniform in viability, and, moreover, that their viabilities at the two temperatures are positively correlated.

Macroenvironmental variability in homozygotes and heterozygotes: The problem which now logically arises concerns the relationships between the viability of homozygotes, or of heterozygotes, for certain chromosomes and their modifiability by environmental influences. Does sensitivity to environmental changes usually go together with low fitness? And do genotypes which show high fitness in one environment tend to preserve this fitness over a range of environments?

We shall examine this problem both in connection with the macroenvironmental and with the microenvironmental variations. To start with the former, as shown in Figures 1 and 2, we have data on the viabilities at 25° and 15°C, as measured by the percentages of the +/+ class of flies produced in the test cultures, of an array of homozygotes and of heterozygotes for certain chromosomes. Let P_{25} and P_{15} be the percentages of +/+ observed at the two temperatures in the test cultures for a given chromosome. We now compute $(P_{25} + P_{15})/2$, which is the unweighted mean percentage of the +/+ class at the two temperatures. Let

this value be called the mean viability, P_m, of a given homozygote or heterozygote. The crude variance of the mean viability is evidently $(P_{25} - P_{15})^2/2$. A part of this variance is certainly due to sampling errors. The sampling variance is easily estimated for P_{25} and for P_{15} from the numbers of the flies counted in the cultures. Subtracting the sum of the sampling variances from the crude variance, we obtain an estimate of the macroenvironmental variance. From this value we derive, further, a coefficient of variation, C_{ma}; this is, evidently, the square root of the macroenvironmental variance, multiplied by 100, and divided by P_m.

The coefficients of variation, C_{ma}, are plotted against the mean viabilities, P_m, in Figures 3 and 4, for homozygotes and heterozygotes respectively. In preparing these diagrams, we ignored the homozygotes which produced fewer than five percent of +/+ flies in the cultures. Where the estimates of C_{ma} had negative

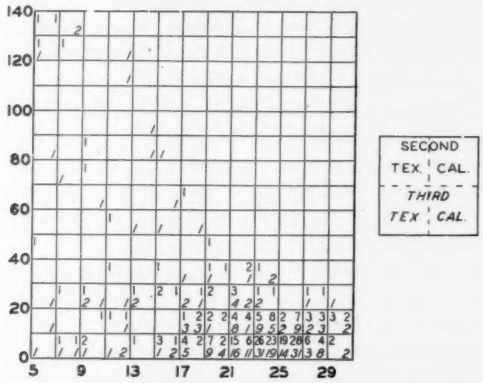

FIGURE 3.—Correlating the viability (abscissae) and the macroenvironmental variability (ordinates) of the homozygotes for different chromosomes. The viability is measured in mean percentages of the wild type class obtained in the cultures raised at 15°C and at 25°C. The figures for the second and the third chromosomes of Texas and of California origins are placed as indicated in the diagram on the right.

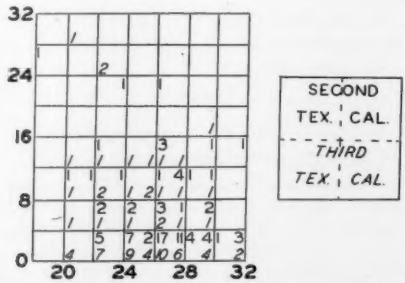

FIGURE 4.—Correlating the viability and the macroenvironmental variability of the heterozygotes for different combinations of chromosomes. This figure is constructed like Figure 3.

signs, they were taken to be zero; (such negative values arise when the crude variance happens to be smaller than the sampling variance). From the data in Figures 3 and 4 we have computed the correlation coefficients, r, which are reported in Table 5. The correlations are negative in every instance. This means that the genotypes which yield high average viabilities tend to be less sensitive to environmental modifications than genotypes which yield lower average fitness. In other words, a genotype which produces good viability at one temperature usually does so, also, at the other temperature; a genotype which is poorly viable at one temperature may be as bad, or better, or worse at the other temperature.

Since none of the heterozygotes in our material are lethal, semilethal, or low subvital, it is reasonable that the correlation coefficients for the heterozygotes are lower than those for the homozygotes. In fact, some of the former are not even significantly different from zero (Table 5). To obtain estimates for homozygotes comparable with those for heterozygotes, we ignored all chromosomes which yielded fewer than 20 percent of +/+ at either temperature, 25° or 15°C. We have thus rejected not only lethal and semilethal but also a number of quasi-normal chromosomes which happen to be clearly subvital at at least one temperature. The correlation coefficients for the remainder of the quasi-normal chromosomes are given in the middle column of Table 5. They are again all negative, although none of them taken separately are high enough to be statistically significant. We conclude that low viability is correlated, both in homozygotes and in heterozygotes, with a sensitivity to temperature changes, and high viability with a capacity to live well at different temperatures.

Microenvironmental variability in homozygotes and heterozygotes: With only few accidental exceptions, the homozygotes for every chromosome, and every heterozygous combination of two chromosomes, were tested in three replicate cultures. The percentages of the +/+ class observed in the replicate cultures vary because of the sampling errors, and also because of the minor variations in the environmental conditions in these cultures (degree of crowding, amount of food, accidental contamination with different microorganisms, etc.). We wish to examine and to compare the microenvironmental variances in the cultures in which the +/+ class consisted of homozygotes and in those where this class was heterozygous for pairs of chromosomes.

The treatment of the microenvironmental variance is quite analogous to that described above for the macroenvironmental variance. First, the observed, or

TABLE 5

Correlations between the mean viability, P_m, and the coefficient of macroenvironmental variation, C_{ma}. (Further explanation in text)

Chromosome	Locality	All homozygotes	Quasi-normal homozygotes	Heterozygotes
Second	Texas	−0.60±0.06	−0.12±0.10	−0.24±0.13
Second	Calif.	−0.45±0.08	−0.18±0.10	−0.33±0.16
Third	Texas	−0.47±0.07	−0.16±0.10	−0.25±0.14
Third	Calif.	−0.61±0.06	−0.16±0.10	−0.10±0.20

crude, variance is computed for each chromosome homozygote or heterozygote. Then the sampling component is estimated from the numbers of the flies counted in the cultures. Subtracting the sampling variance from the crude variance gives an estimate of the environmental variance. Where this estimate comes out negative, it is considered to equal zero. The standard deviations and the coefficients of microenvironmental variance, C_{mi}, are then calculated. In Figures 5 and 6 these coefficients are plotted against the mean viabilities (P_{25} or P_{15}), as measured by the percentages of the +/+ class among the flies counted in a given group of three replicate cultures.

The correlation coefficients, r, computed from the data reported in Figures 5 and 6, are presented in Table 6. For the homozygotes, the eight r's are all negative, and all but one are statistically significant. It is evident that, as with the macroenvironmental variability, the microenvironmental variability tends to be relatively greater in poorly viable homozygotes than in homozygotes of better viability. The situation is not so clear for the heterozygotes, since here three of the eight r's are ostensibly positive, and only one (negative) r is significantly

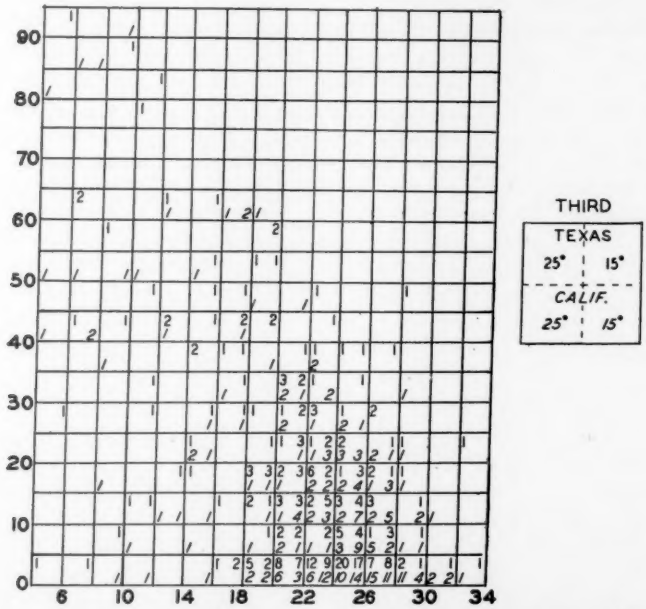

FIGURE 5.—Correlating the viability (abscissae) and the microenvironmental variability (ordinates) of the homozygotes for different third chromosomes. The viability is measured by the mean percentages of the wild type class observed in the cultures, and the variability by the coefficients of variation between the replicated cultures raised at the same temperature. The figures for the chromosomes of different geographic origins are placed as shown in the diagram on the right.

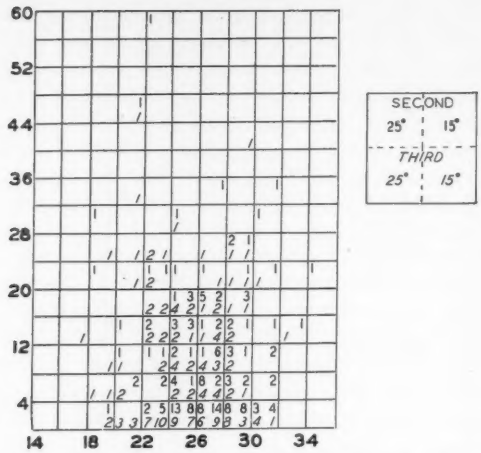

FIGURE 6.—Correlating the viability and the microenviromental variability of the heterozygotes for different chromosomes. This figure is constructed like Figure 5.

TABLE 6

Coefficients of correlation between the mean variability at a given temperature (P_{25} or P_{15}) and the coefficient of microenvironmental variation, C_{mi}. (Further explanation in text)

Chromosome	Locality	t°C	All homozygotes	Quasi-normal homozygotes	Heterozygotes
Second	Texas	25°	—0.11	—0.08	—0.19
Second	Texas	15°	—0.36	+0.01	—0.10
Second	Calif.	25°	—0.49	—0.25	—0.14
Second	Calif.	15°	—0.25	—0.10	—0.00
Third	Texas	25°	—0.66	+0.02	—0.30
Third	Texas	15°	—0.59	—0.26	+0.18
Third	Calif.	25°	—0.49	—0.24	+0.06
Third	Calif.	15°	—0.59	—0.25	+0.00

different from zero. To obtain comparable data, we have computed the correlation coefficients only for those quasi-normal homozygotes which yielded more than 20 percent of +/+ flies in the sum of the three replicated cultures. The figures are given in the column second from the right in Table 6. Two of the eight r's are positive (but neither of them is significantly different from zero), and six are negative (four of them close to the conventional significance level).

It may, then, be that the microenvironmental variability tends to be lower in heterozygotes than in homozygotes of comparable mean viability. To test this inference further we have calculated the mean coefficients of microenvironmental variability for the quasi-normal homozygotes (yielding more than 20 percent of +/+ in the cultures), and for the heterozygotes. The data are summarized in Table 7. It can be seen that the mean C_{mi} is ostensibly higher for the quasi-normal

TABLE 7

Mean coefficients of microenvironmental variation (C_{mi}) for the quasi-normal homozygotes and for the heterozygotes

Chromosome	Locality	t°C	Quasi-normal homozygotes	Heterozygotes
Second	Texas	25°	12.17	8.92
Second	Texas	15°	8.14	6.96
Second	Calif.	25°	9.53	8.13
Second	Calif.	15°	10.30	9.06
Third	Texas	25°	11.40	8.22
Third	Texas	15°	10.35	10.16
Third	Calif.	25°	9.55	5.54
Third	Calif.	15°	9.51	6.84

homozygotes than for the heterozygotes in six out of the eight comparisons. However, the difference is significant only in two comparisons (the third chromosomes tested at 25°C, in both of which the homozygotes are more variable. Taking all the data together, the mean C_{mi} for the quasi-normal homozygotes turns out to be 9.813 and for the heterozygotes, 8.259; the difference between these two figures is 1.554 ± 0.624, which, by the ordinary t test, is significant between the 0.02 and 0.01 probability levels. We conclude that chromosome homozygotes show, on the average, greater microenvironmental variabilities than the heterozygotes of similar viability.

Are heterozygous flies phenotypically uniform?: Drosophila pseudoobscura is a widespread and common species often forming populations of rather large genetically effective sizes (DOBZHANSKY and WRIGHT 1941, 1947, and other works). In populations of this sort, individuals homozygous for any one chromosome may be expected to occur only rarely; a great majority of the wild flies are heterozygotes. How the homozygosis for a given chromosome affects the fitness of its carriers is, then, of relatively minor importance in determining the fate of this chromosome, and the fate of the genes which it carries, under natural selection. On the contrary, the variations in fitness, even minor ones, which a chromosome might produce in its heterozygous carriers will be quite important determinants of its retention in, or elimination from, the gene pool of the population. One looks, therefore, for evidence of variation in fitness among the heterozygotes which carry different chromosomes.

Relevant evidence has been presented in Table 4. If the viability of the wild type heterozygotes were uniform, then the variance of the percentages of the +/+ class observed in the control experiments would be entirely accountable for by environmental variations and sampling errors; the genetic variance, σ_g^2, would be zero. Indeed, Table 4 shows that three of the eight estimates of σ_g^2 are negative, but the mean of the eight is still positive, +0.44. This may not be significantly different from zero; the average σ_g^2 for homozygotes is about 15 times larger and quite significant. Nevertheless, we have good reasons to think that the σ_g^2 for heterozygotes is greater than zero. In Figures 1 and 2 we have plotted the per-

centages of the +/+ class which a given chromosome or chromosome combination yielded at the two temperatures, 25° and 15°C. (The correlation coefficients computed from such data have been presented on a preceding page.) These correlation coefficients are positive and vary from $+0.16 \pm 0.14$ to $+0.31 \pm 0.09$, two of them being statistically significant even when considered in isolation from the others. Now, if the variance of the percentages of the +/+ class obtained at either temperature were due wholly to sampling errors and to microenvironmental variations, then no correlation between the results at the two temperatures would be expected. In reality, this correlation is not much weaker for the heterozygotes than it is for the quasi-normal homozygotes. We conclude that genetically conditioned variations in the viability do exist among the "wild type" heterozygotes as they do, to a much greater extent, among the homozygotes.

Comparing the productivity of the cultures in which homozygotes and heterozygotes are born: The data presented above show that the viability of individuals homozygous for second and third chromosomes extracted from natural populations is lower than that of the "normal" heterozygotes. We wish to inquire whether the productivity of (the number of flies hatching in) experimental test cultures which produce about one third or less of the progeny consisting of homozygotes falls below that of the cultures which produce heterozygotes.

Two facts must be kept in mind in this connection. First, the "wild type" heterozygotes and homozygotes are both produced in cultures in which a majority of the flies are heterozygous for a wild chromosome and for a chromosome with mutant marker genes. Secondly, our experimental cultures are deliberately somewhat overpopulated in order to help bring out the viability variations. Some mortality doubtless takes place in these cultures, and only a part of the eggs deposited survive to become adult flies. It would not be very surprising, therefore, if we were to find that even the cultures in which the homozygous class is eliminated by lethality would not suffer any diminution of the productivity. The data summarized in Table 8 show, however, that such a diminution does occur.

Excepting the California second chromosomes tested at 15°C, every one of the

TABLE 8

Number of flies in test cultures in which the +/+ class is homozygous or heterozygous for certain chromosomes

			Homozygotes			Heterozygotes		
Chromosome	Locality	t°C	Chromosomes tested	Total flies	Flies per chromosome	Combinations tested	Total flies	Flies per combination
Second	Texas	25°	156	87,103	558.4	50	31,106	622.1
Second	Texas	15°	154	99,785	648.0	52	35,306	679.0
Second	Calif.	25°	132	76,035	576.0	34	20,531	603.9
Second	Calif.	15°	130	77,395	595.3	33	19,615	594.4
Third	Texas	25°	153	77,600	507.2	56	32,847	586.5
Third	Texas	15°	152	87,474	575.5	51	29,953	587.3
Third	Calif.	25°	140	78,341	559.6	26	16,722	643.2
Third	Calif.	15°	140	82,129	586.6	26	15,506	596.4
Total			1,157	665,862	575.5	328	201,586	614.6

seven possible comparisons in Table 8 show that the cultures in which the homozygotes are produced contain fewer flies than do cultures in which the "wild type" class is heterozygous. The mean numbers of flies per tested chromosome, or chromosome combination, are 575.5 for the homozygotes and 614.6 for the heterozygote-producing tests. Since every test involved raising three replicate cultures (see above), the average numbers of flies per culture were 191.8 and 204.9, respectively. This means a loss of about 6.4 percent of the productivity of the cultures, owing to the deficient viability of the homozygous class.

SUMMARY

The aim of the present work has been to estimate the magnitude of the genetic loads carried in the second and third chromosomes of *Drosophila pseudoobscura*. Samples of 156 second and 153 third chromosomes from the population living near Austin, Texas, and 132 second and 140 third chromosomes from Mather, California, have been studied. The effects of homozygosis for these chromosomes were examined in flies raised at 25° and at 15°C.

Homozygosis for second chromosomes results in a loss of, on the average, between one quarter and one third of the viability of homozygotes (compared to an average heterozygote). The average viability loss in homozygotes for third chromosomes is slightly smaller, 21 to 26 percent. There is no great difference in the magnitudes of the losses at the near-optimal temperature 15°C and at the less favorable 25°C.

Between 21.5 and 25.6 percent of the second, and between 16.4 and 22.9 percent of the third chromosomes in our samples were lethal or semilethal when in double dose. Among the chromosomes free of lethals and semilethals, roughly 78 percent of the second and 61 percent of the third chromosomes produced subvital homozygotes.

The effects of the chromosomes on the viability of the homozygotes are often different in the cultures raised at 25° and at 15°C. There is, nevertheless, a fairly strong correlation between the viability records at the two temperatures (Figure 1).

It is convenient for our purposes to distinguish between macro- and microenvironmental variability. The former concerns the performance of the chromosomes at the two temperatures, 15° and 25°C, and the latter refers to variations observed in replicate cultures at the same temperature. Both the macro- and the microenvironmental variabilities, as measured by the coefficients of variation, are greater in the less viable than in the more viable phenotypes. In other words, there is a negative correlation between the viability and the variability. This is true both for homozygotes and for heterozygotes. However, the mean variation coefficients are somewhat lower for heterozygotes than for homozygotes of about equal viability (Table 7). A superior viability record in one environment is, thus, usually accompanied by good records in other environments. Highly viable homozygotes and heterozygotes are those which possess superior homeostatic properties, and the homeostatic capacities are on the average higher in heterozygotes than in homozygotes.

ACKNOWLEDGMENTS

It is a pleasure to acknowledge our obligations to PROF. HOWARD LEVENE and to PROF. BRUCE WALLACE for their invaluable advice concerning the statistical treatment of the data reported in the present article, and for the many discussions of the plans and the results of this investigation.

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GENETICS OF NATURAL POPULATIONS. XXIX. IS THE GENETIC LOAD IN DROSOPHILA PSEUDOOBSCURA A MUTATIONAL OR A BALANCED LOAD?

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THE foregoing paper (B. SPASSKY, N. SPASSKY, PAVLOVSKY, M. G. KRIMBAS, C. KRIMBAS, and DOBZHANSKY 1960) describes estimates of the magnitude of the genetic loads carried in the second and third chromosomes of populations of *Drosophila pseudoobscura* from Texas and California. We are now ready to consider the problem of the origin and maintenance of these loads. Two principal components may be distinguished in genetic loads. The mutational component consists of genetic variants which reduce the fitness of their homozygous, or of both homozygous and heterozygous carriers, in most environments which the population encounters. The maintenance in populations of mutational loads is due primarily to mutation pressure. The balanced component of the load consists of heterotic (overdominant) genes and gene complexes, which yield highly fit heterozygotes but relatively less well adapted homozygotes. Balanced loads are maintained primarily by natural selection.

The biological (and sociological, in the case of human populations) meaning and function of the mutational and the balanced components of the genetic loads are profoundly different. Mutational loads are regarded, by some authors, as unavoidable evil consequences of the occurrence of mutation, and of the fact that most mutants are more or less harmful in, at least, the "normal" environment of the species. Balanced loads are adaptively ambivalent (deleterious to homozygous carriers but favorable to heterozygotes); under certain conditions they may function as mechanisms of adaptation to the environment. Unfortunately, it is not at all simple to discriminate between, and to evaluate the relative magnitudes of, the mutational and balanced components of the loads. A most ingenious method devised for human populations, by MORTON, CROW, and MULLER (1956) and CROW (1958) is based on some unproved assumptions which make its results unconvincing (DOBZHANSKY 1958). In materials, such as Drosophila, which lend themselves to genetic experimentation, rather more direct approaches are feasible. Our attempts to use these approaches are described in the pages that follow, but the reader is hereby warned at the outset that the results obtained are still inconclusive.

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[2] Research Fellow of the International Cooperation Administration.

THE EXPERIMENTS

Descriptions of the material used and of the experimental procedures have been given by SPASSKY *et al.* (1960), and will not be repeated here. What is necessary to keep in mind is that the experiments consisted in intercrossing *Ba gl Inv*/+ females to Δ/+ males (for the analysis of the second chromosomes), or of *L or Inv*/+ females to *Bl Sc pr*/+ males (for the analysis of the third chromosomes). The progenies consisted of the following classes:

Wild type (+/+)		Wild type (+/+)
Bare (*Ba gl Inv*/+)	or:	Blade-Scute (*Bl Sc pr*/+)
Delta (Δ/+)		Lobe (*L or Inv*/+)
Bare-Delta (*Ba gl Inv*/Δ)		Blade-Scute-Lobe (*Bl Sc pr*/*L or Inv*)

In some of the tests the parents intercrossed contained the same wild (+) chromosome, and accordingly the wild type class in the progeny (+/+) contained this chromosome in duplicate (homozygotes). In other tests (controls) the parents had chromosomes derived from different wild progenitors, and then the +/+ progeny were heterozygotes. The Bare, Delta, Blade-Scute, and Lobe classes were in all cases heterozygous carriers of wild chromosomes. And finally the Bare-Delta and Blade-Scute-Lobe classes were free of the wild chromosomes under test. Almost all tests were run at 25°C and at 15°C, and three replicate cultures were raised at each temperature.

The viability of the +/+ class can be measured either in percentages of individuals of this class among the total number of the flies produced in a culture or cultures, or in ratios of +/+ to Bare-Delta, or to Blade-Scute-Lobe flies, respectively. The ratios exhibit greater variances than the percentages, and consequently the percentages were used throughout by SPASSKY *et al.* (1960). For the purposes of the present study both percentages and ratios will be used. The advantage of the latter is that, since the Bare-Delta and Blade-Scute-Lobe classes do not contain the wild chromosomes to be tested, these classes serve as common denominators making the different tests comparable.

Absence of perceptible correlations between the viabilities of the +/+ homozygotes and heterozygotes: CORDEIRO and DOBZHANSKY (1954) have shown that the viability of heterozygous "wild type" flies, +/+, of *Drosophila willistoni* depends upon the particular combination of "normal" or "wild" chromosomes which these flies carry. SPASSKY *et al.* (1960) have demonstrated the existence of such genetically determined variations in fitness also in *D. pseudoobscura*. In a way, this may be regarded as self-evident and in no need of demonstration. After all, "normal" humans also vary in health and in vigor, and these variations are in part genetically conditioned. It is, however, useful to stress that "normal" men and "wild type" flies are not all alike, that there is no such thing as the "type" of the species, race, or population, and that all such expressions are only loosely descriptive, referring to the absence of strikingly apparent major mutants and abnormalities. No one genotype will yield highest fitness in all environments, or in all occupations or social roles, which the population encounters.

Whether the viability of fitness of homozygous carriers of a given chromosome

is correlated with that of its heterozygous carriers is an entirely different question. One of the ways of approaching this problem from our data is to investigate the correlation between the frequencies of the +/+ class in test cultures in which this class is homozygous for a given chromosome, and in cultures in which it carries the same chromosome in combination with some other chromosome. The data are summarized in the form of two correlation tables in Figure 1. The two variables are the percentage of +/+ flies in test cultures heterozygous for two different wild chromosomes, and the mean of the percentages of such flies in cultures homozygous for each of these two chromosomes. The correlation coefficients, r, calculated from these data are:

Second chromosomes at 25°C	+0.03
Second chromosomes at 15°C	+0.19
Third chromosomes at 25°C	+0.04
Third chromosomes at 15°C	−0.03

None of these correlation coefficients is anywhere near significantly different from zero.

Homozygous and heterozygous classes in the main experiment: Correlating the viabilities of the +/+ classes in the homozygous and heterozygous cultures

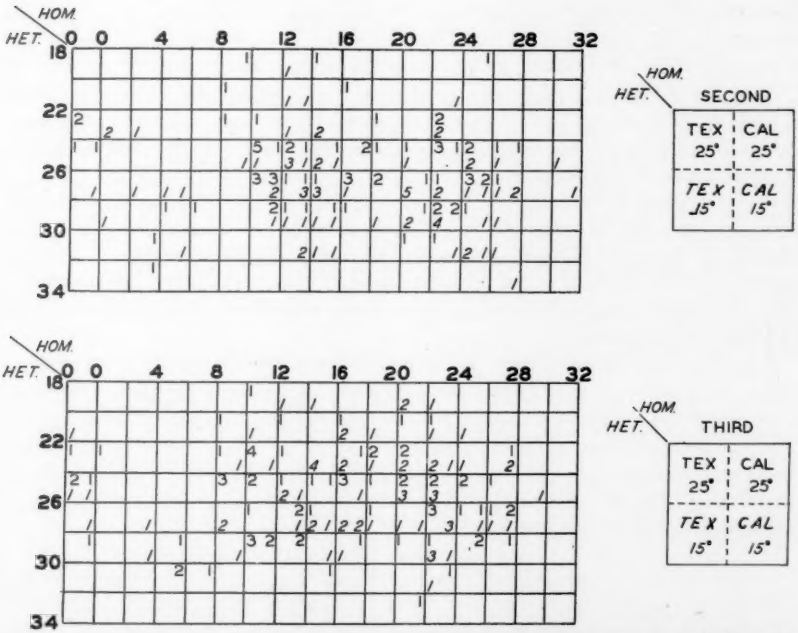

FIGURE 1.—Viability of the wild type homozygotes (as measured in percentages in the main experiments) and of the wild type heterozygotes (as measured in the control experiments) for certain chromosomes. The squares on the right indicate the locations of the figures in the tables.

would seem to be the most straightforward test of the existence of dominant or overdominant viability effects of the wild chromosomes. As shown above, the outcome of this test is inconclusive, because only rather limited numbers of the chromosomes were tested in both homozygotes and heterozygotes in our experiments. Considerably more abundant data are available in the tests in which the +/+ class is homozygous, but in which the Bare, Delta, Blade-Scute, and Lobe classes are heterozygous for certain wild chromosomes.

Statistical analysis of the latter type of data is, however, beset with pitfalls. Suppose that we correlate the observed percentages of the +/+ class with those of the Bare, Delta, Blade-Scute, or Lobe classes in the same cultures. A negative correlation will be obtained, which may be wholly spurious, since an increase in the percentage of one class in a culture automatically depresses the percentages of the other classes, and *vice versa*. One might also correlate the observed ratios of the +/+ to Bare-Delta classes with those of Bare:Bare-Delta flies in the cultures. This gives a spurious positive correlation, since both ratios will be increased in cultures with few, and decreased in those with many Bare-Delta flies.

An avenue of escape from the above difficulties is fortunately opened by the fact that almost all tests in our experiments were made in three replicate cultures. Suppose, then, that we wish to know whether the viability of the homozygotes, +/+, for a given chromosome is correlated with that of, for example, the Delta/+ heterozygotes carrying the same wild (+) chromosome. The frequencies of the wild type, Delta, and Bare classes in the cultures are expressed as ratios of the numbers of the observed individuals of these classes, divided by number of Delta-Bare individuals plus one (HALDANE's correction, see WALLACE 1956; HALDANE 1956) in the same cultures. Since we have three replicate cultures for each test, we may compare the frequency of wild type flies in the culture A with those of Delta in cultures B and C, of wild type in B with Delta in A and C, and of the wild type in C with Delta in A and B. Correlation tables were then constructed. For each chromosome tested in three replicate cultures six points were entered in the table. There may be a bias, though a slight one, even in correlation tables so constructed. It is removed, however, by using the correcting procedure devised for our data by PROFESSOR LEVENE and described more fully in the final section on correlations.

Let v_{ij} be the ratio of $+^i/+^i$: Delta-Bare flies in the jth bottle of the ith chromosome, and w_{ij} the ratio of Delta:Delta-Bare flies in the same bottle. Let z_{ij} be the number of the Delta-Bare flies in the jth bottle of the ith chromosome. Also let $\bar{z}$ be the over-all mean of the z_{ij}'s, and let $\bar{w}$, $\bar{v}$, s_w^2 s_v^2 $s_{w,v}$, $r = r_{w,v}$ be the means, variances, covariance, and correlation coefficient calculated directly from the correlation table described above. Then the corrected correlation coefficient, r_c is simply r multiplied by a correction factor,

$$r_c = gr$$

where

$$g = \frac{\bar{z} - 1}{\sqrt{\left(\bar{z} - \frac{\bar{w} + \bar{w}^2}{s_w^2}\right)\left(\bar{z} - \frac{\bar{v} + \bar{v}^2}{s_v^2}\right)}}$$

The standard error of r_c is then approximately.

$$\sigma(r_c) = g(1 - r^2)/\sqrt{n},$$

or simply the correction factor, g, times the standard error of the uncorrected r. It should be noted that n is the number of different wild chromosomes tested, which is only about one sixth of the number of entries in the correlation table.

For testing whether the true correlation is zero, r in the variance formula is set equal to zero, and the test is equivalent to comparing the uncorrected r with its standard error, $1/\sqrt{n}$.

Representative correlation grids are shown in Figures 2 and 3. The correlation coefficients calculated from these data are reported in Table 1, including both the uncorrected coefficients of correlation and the ones corrected as indicated above, together with their standard errors. It can be seen that the correction changes the correlation coefficients only slightly, always making them a little larger numerically. Another precaution has also been taken. The raw data show that some scattered cultures have produced rather few flies, especially in the classes (Bare-Delta and Blade-Scute-Lobe) which serve as divisors for the calculation of the frequency ratios of the other classes (see above). The frequency ratios in such cultures are obviously subject to considerable errors. Therefore, the calculations were made in two ways; first, all the cultures were included in the calculations, and secondly, the cultures which had small numbers, fewer than ten, of the Bare-Delta or Blade-Scute-Lobe flies were disregarded. The numbers of the chromosomes tested are shown in the columns marked "N." It may be seen that only

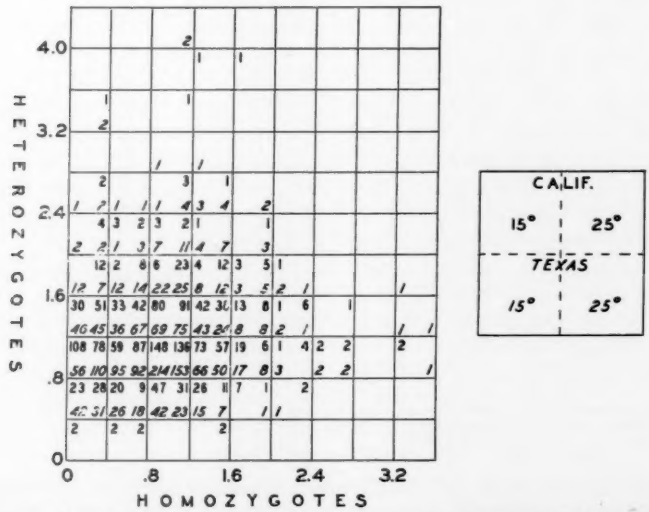

FIGURE 2.—Correlation between the frequency ratios of the homozygotes for different wild second chromosomes and the frequency ratios of the Bare heterozygotes for the same chromosomes. (Further explanation in text).

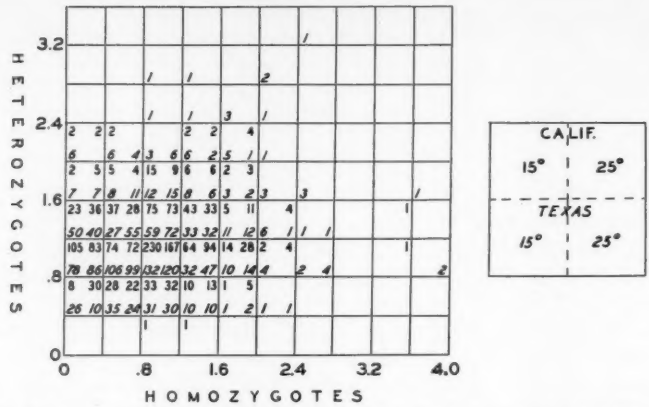

FIGURE 3.—Correlation between the frequency ratios of the homozygotes for different wild third chromosomes and the frequency ratios of the Blade-Scute heterozygotes for the same chromosomes. (Further explanation in text).

TABLE 1

Correlations of the frequencies of the homozygous (+/+) and of the heterozygous classes in the cultures

Chromosome	Locality	t°C	Gene marker	All cultures			Excluding small		
				Uncorrected	Corrected	N†	Uncorrected	Corrected	N
Second	Texas	15	*Ba*	0.11	0.14±0.11	147	0.11	0.15±0.11	147
Second	Texas	15		0.12	0.16±0.11	147	0.12	0.16±0.11	147
Second	Texas	25	*Ba*	0.17	*0.19±0.09	152	0.17	*0.21±0.10	146
Second	Texas	25		0.08	0.10±0.10	152	0.08	0.11±0.11	146
Second	Calif.	15	*Ba*	0.14	0.18±0.11	130	0.10	0.14±0.12	130
Second	Calif.	15		0.11	0.15±0.11	130	0.07	0.10±0.12	130
Second	Calif.	25	*Ba*	0.06	0.08±0.12	131	0.04	0.06±0.12	131
Second	Calif.	25		—0.02	—0.03±0.12	131	—0.02	—0.03±0.17	131
Third	Texas	15	*BlSc*	0.31	*0.38±0.10	135	0.28	*0.34±0.10	131
Third	Texas	15	*L*	0.31	*0.34±0.09	135	0.28	*0.34±0.10	131
Third	Texas	25	*BlSc*	0.02	0.03±0.11	137	0.04	0.07±0.19	131
Third	Texas	25	*L*	0.06	0.07±0.10	137	0.08	0.10±0.11	131
Third	Calif.	15	*BlSc*	0.10	0.17±0.14	138	0.08	0.12±0.14	136
Third	Calif.	15	*L*	0.04	0.07±0.13	138	0.02	0.03±0.14	136
Third	Calif.	25	*BlSc*	0.04	0.06±0.12	137	0.09	0.14±0.14	136
Third	Calif.	25	*L*	0.09	0.12±0.12	137	0.08	0.12±0.12	136
		Means		0.11	0.14		0.10	0.13	

* The correlation coefficients which reach the level of statistical significance.
† N—The numbers of chromosomes examined.

a few chromosomes have been lost owing to the exclusion of the small cultures.

Inspection of Table 1 shows that the correlation coefficients are mostly positive but not significantly different from zero. However, among the 16 correlation coefficients computed taking all cultures into account only three reach the con-

ventional level of statistical significance, being more than twice their standard errors. Exclusion of the cultures with small numbers of the Bare-Delta or Blade-Scute-Lobe flies sometimes ostensibly decreases, and sometimes increases, the correlation coefficients. However, only the same three correlation coefficients are significant when the small cultures are ignored.

The results are, nevertheless, not as ambiguous as they may seem at first glance. Some of the correlation coefficients are positive and statistically significant according to the conventional criterion of significance. Moreover, 15 out of the 16 correlations turn out to have positive signs, and only one has a negative sign. Some positive correlation between the viabilities of the homozygotes and the heterozygotes for the chromosomes studied does exist. The mean of the corrected coefficients of correlation is about +0.13. Taken at face value, this is consistent with the surmise that the mutational component of the genetic load is more important than the balanced component. Further analysis is, however, needed.

Recessivity of lethals in the control experiments: STERN, CARSON, KINST, NOVITSKI, and UPHOFF (1952) showed that the viability of heterozygotes for sex-linked lethals in *D. melanogaster* is *on the average* lower than that of lethal-free heterozygotes. STERN and his collaborators have, however, pointed out that some lethals caused no viability losses when heterozygous, and a few seemed to be heterotic. Regrettably, the data of STERN *et al.* (1952) have often been quoted as showing a universal inferiority of heterozygotes for lethals. The work of CORDEIRO (1952) and of PROUT (1952) on naturally-occurring autosomal lethals in *D. willistoni* led to conclusions similar to those of STERN *et al.* CORDEIRO and DOBZHANSKY (1954) demonstrated further that the effects of heterozygosis for a lethal on the viability of its carriers depend, in *D. willistoni*, on the genetic background on which the lethal is placed. The same lethal may be deleterious in some and heterotic in other genotypes. WALLACE (1957, 1958, 1959a,b) and BURDICK and MUKAI (1958) approached the problem more broadly; they showed that a considerable proportion of mutants which are deleterious (but not necessarily lethal) in homozygotes are heterotic in heterozygotes (in *D. melanogaster*).

Table 2 reports data on the viability of comparable lethal-free and lethal-carrying heterozygotes. The data have been obtained as follows. In our "control" experiments we intercrossed strains containing different wild chromosomes, so that the flies of the +/+ class obtained in the progeny were heterozygotes (SPASSKY *et al.* 1960). In the "main" experiment the viability of the same chromosomes in homozygotes was investigated. We divided the chromosomes into lethal-free ones, and those which were lethal or semilethal when homozygous. The mean frequencies of the various heterozygotes free of lethals or carrying a lethal are shown in Table 2, with their standard errors.

There is no trace of an incapacitation of heterozygotes by the lethals. In no instance are the means for lethal-free and lethal-carrying heterozygotes significantly different. Among the 12 possible comparisons (the data for Texas and California chromosomes combined), the mean for the lethal-free heterozygotes is ostensibly higher than that of the lethal-carrying ones in five cases, ostensibly lower in five cases, and equal in two cases.

TABLE 2

Mean viabilities of heterozygotes carrying a chromosome which is lethal or semilethal when homozygous, compared with heterozygotes for chromosomes yielding normally viable homozygotes

Chromosome	t°C	Gene marker	Lethal free	Carrying lethal
Second	25	*Ba*	1.14±0.03	1.14±0.03
Second	25	Δ	1.01±0.03	1.05±0.03
Second	25	+	1.16±0.03	1.12±0.03
Second	15	*Ba*	1.04±0.02	1.06±0.02
Second	15	Δ	1.08±0.03	1.12±0.03
Second	15	+	1.17±0.03	1.15±0.03
Third	25	*BlSc*	1.07±0.03	1.08±0.02
Third	25	*L*	1.09±0.03	1.05±0.03
Third	25	+	1.11±0.03	1.09±0.03
Third	15	*BlSc*	1.10±0.03	1.05±0.03
Third	15	*L*	1.00±0.03	1.00±0.02
Third	15	+	1.02±0.03	1.04±0.03

Recessivity of lethals in the main experiment: Are the different results reported above mutually compatible? A slight but positive correlation between the viability of the homozygotes and heterozygotes has been shown to exist, and yet the lethal-carrying and the lethal-free heterozygotes seem to be equally viable. What kind of chromosomes are responsible for the positive correlation? The relevant data are presented in Table 3. We have divided the chromosomes into eight classes according to their viabilities in the homozygotes, starting with the lethals and extreme semilethals (0–0.2), the less extreme semilethals (0.2–0.4), subvitals (0.4–0.8), normals (0.8–1.2), and the ostensible supervitals (>1.2). The mean frequency ratios of the heterozygous classes in the same cultures were then calculated.

Consideration of the data in Table 3 may be started with the column means. The means are 1.12 for lethals and extreme semilethals, 1.14 for less extreme semilethals, 1.09 and 1.11 for subvitals, and 1.14 for the chromosomes normally viable when homozygous. There is evidently no trace of a positive regression. However, the supervitals give higher values—1.17 and 1.25. It seems, then, that the chromosomes which make the homozygotes ostensibly supervital increase the viability of the heterozygotes as well, but other chromosomes have no systematic effects in heterozygotes.

This conclusion is upheld by examination of the separate experiments on chromosomes from different localities tested at the two temperatures. Consider the chromosomes which give frequency ratios of the homozygotes above 1.14 (supervitals). The frequency ratios of the heterozygotes for these chromosomes (the rightmost column of figures in Table 3) are higher than those of any other class of chromosomes in ten out of the 16 cases observed (the other columns of figures in Table 3). Contrast this with the behavior of the lethal and extreme semilethal chromosomes (the leftmost column of figures in Table 3). The frequency ratios

TABLE 3

Mean viabilities of the heterozygotes carrying one wild chromosome and one chromosome with a mutant gene marker, for different viability classes of the homozygotes for the same wild chromosomes. (Further explanation in text)

Chromosome	Locality	t°C	Gene marker	Viabilities of the homozygotes 0	0.2	0.4	0.6	0.8	1.0	1.2	1.4	>1.4
Second	Texas	15	*Ba*	1.04	1.26	1.08	1.11	1.10	1.13	1.21	1.20	
Second	Texas	15	Δ	1.08	1.24	1.15	1.10	1.16	1.19	1.21	1.21	
Second	Texas	25	*Ba*	1.26	1.13	1.19	1.19	1.24	1.23	1.24	1.47	
Second	Texas	25	Δ	1.03	1.13	1.16	1.08	1.10	1.10	1.14	1.19	
Second	Calif.	15	*Ba*	1.02	1.08	1.03	1.10	1.09	1.07	1.11	1.13	
Second	Calif.	15	Δ	1.12	1.11	1.15	1.15	1.14	1.14	1.19	1.19	
Second	Calif.	25	*Ba*	1.16	1.22	1.10	1.17	1.25	1.11	1.16	1.24	
Second	Calif.	25	Δ	1.12	1.13	1.08	1.10	1.15	1.07	1.05	1.14	
Third	Texas	15	*BlSc*	1.13	1.11	1.05	1.05	1.10	1.15	1.26	1.51	
Third	Texas	15	*L*	1.15	1.16	1.07	1.08	1.10	1.21	1.34	1.60	
Third	Texas	25	*BlSc*	1.11	1.18	1.11	1.13	1.18	1.15	1.17	1.16	
Third	Texas	25	*L*	1.17	1.23	1.05	1.18	1.23	1.28	1.19	1.29	
Third	Calif.	15	*BlSc*	1.09	1.01	1.05	1.07	1.10	1.08	1.14	1.15	
Third	Calif.	15	*L*	1.09	0.99	1.07	1.02	1.04	1.04	1.07	1.07	
Third	Calif.	25	*BlSc*	1.08	1.07	1.02	1.06	1.06	1.10	1.07	1.18	
Third	Calif.	25	*L*	1.19	1.18	1.03	1.15	1.14	1.24	1.17	1.25	
		Means		1.12	1.14	1.09	1.11	1.14	1.14	1.17	1.25	

of the heterozygotes for these chromosomes are the lowest in only four among the 16 possible comparisons.

Figure 4 shows a regression line calculated from all the column means given in Table 3. The observed points do not follow this line. The regression in our ma-

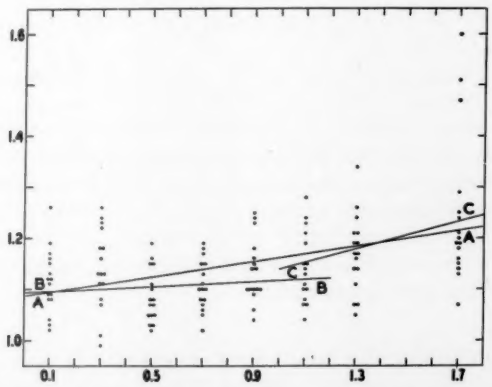

FIGURE 4.—Correlation of the viabilities of homozygotes (abscissae) and of heterozygotes carrying mutant markers (ordinates) for different wild chromosomes. AA—the regression line including all the data; BB—excluding the chromosomes which act as supervitals when homozygous; CC—excluding the chromosomes which are lethal, semilethal, or subvital when homozygous. Compare with Table 3. (Further explanation in text).

terial is evidently not a rectilinear one. Two other regression lines are also shown in Figure 4, one for the range of the chromosome viabilities extending from lethality (0) to normality (1.0), and the other for normal and supervital chromosomes (1.0 to >1.4). The first of these regression lines is horizontal, while the second has a clear positive slope.

The only previously published data with which our present data may be compared are those of DOBZHANSKY and LEVENE (1955). They investigated ten different heterozygotes which carried two different chromosomes, each of which was known to be normal to supervital when homozygous, nine heterozygotes each carrying one normal to supervital and one subvital chromosomes, and eight heterozygotes carrying two subvital chromosomes. No trace of a systematic difference between these three classes of heterozygotes was found. This result seemed to favor the view that the component of the genetic load consisting of the chromosomes which are mildly detrimental in homozygotes is mainly the balanced component. The present results would not be expected with either balanced or mutational loads. The lethals, semilethals, and subvitals do not harm the heterozygotes; these genetic variants injurious to homozygotes are maintained in the populations by a heterotic mechanism, rather than by mutation pressure. And yet some chromosomes seem to be supervital both when homozygous and when heterozygous; one would expect such chromosomes to crowd out and replace all others in the populations, but there is no evidence that any such process is under way. Very tentatively, we wish to suggest that the apparent supervitality of these chromosomes may be confined to heterozygotes which contain the laboratory mutants which we have used as markers. The possibility of experimental errors is also not excluded, although a careful scrutiny of the records failed to disclose a source to which such errors could be ascribed.

SUMMARY

The question asked in the title of the present article cannot be answered unambiguously. For most of the viability classes, the fitness of the homozygotes for the chromosomes studied shows no positive correlation with that of the corresponding heterozygotes. Not even the lethal chromosomes show any trace of deleterious dominant effects in the heterozygotes. This is what one would expect if most of the genetic load were a balanced load. On the other hand, a minority of the chromosomes which give highly viable homozygotes seem to produce also most viable heterozygotes. This is expected with neither balanced nor mutational loads. The problem remains unsolved.

ACKNOWLEDGMENT

We owe a debt of gratitude to DRS. E. R. DEMPSTER, I. M. LERNER, HOWARD LEVENE, R. D. LEWONTIN, and BRUCE WALLACE for numerous and detailed instructions concerning the statistical analysis of the data.

CORRECTION TO THE CORRELATIONS

HOWARD LEVENE[1]

In a given culture bottle, the frequencies of the four genotypes it contains will have a multinomial distribution. If the customary assumption of a fixed total number of flies in the culture is dropped, then the four frequencies may be assumed to be independently distributed with Poisson distributions. For considering the distribution of ratios of two classes of flies, the two assumptions are equivalent, and the Poisson assumption is more convenient. Then if z_{ij} is the number, say, of Bare-Delta flies in the jth culture involving the ith wild chromosome, z_{ij} will have a Poisson distribution with mean and variance Z_{ij} depending on the environmental conditions of this culture. The number of wild flies, x_{ij}, will have a Poisson distribution with mean X_{ij} depending on the environmental variable Z_{ij} and on the genetic contribution of the $+^i/+^i$ homozygote, t_i. Similarly the number of Delta/$+^i$ flies, y_{ij}, will have mean Y_{ij} depending on the environmental component Z_{ij} and genetic component p_i. The means, Z_{ij}, X_{ij}, and Y_{ij}, of the Poisson variables, z_{ij}, x_{ij}, and y_{ij}, are themselves random variables depending on genetic and environmental components. These may be specified by various mathematical models. Some of these are:

$$(1)\quad Z_{ij} = a_{ij} + b_i,\ \frac{1}{Z_{ij}} = 1/(a_{ij} + b_i),$$

$$(2)\quad \frac{1}{Z_{ij}} = c_{ij} + f_i,\ Z_{ij} = 1/(c_{ij} + f_i),$$

$$(3)\quad Z_{ij} = a_{ij}b_i,\ \frac{1}{Z_{ij}} = c_{ij}f_i,$$

where b_i and f_i represent environmental influences common to all the cultures involving the ith wild chromosome but not all other chromosomes (the cultures involving a given chromosome are usually simultaneous), and a_{ij} and c_{ij} involve environmental conditions specific to a given culture. Model 1 is the most convenient in dealing with Z_{ij}, but is inconvenient for $1/Z_{ij}$, while the reverse is true for model 2; model 3 is moderately convenient for both Z_{ij} and $1/Z_{ij}$, and hence most convenient in general, since both Z_{ij} and $1/Z_{ij}$ appear in the equations. It is not clear which model is most realistic, but all three are nearly correct if the coefficient of variation of Z_{ij} is small. Hence the most convenient model, 3, was used. Then

$$X_{ij} = t_i b_i a_{ij}$$

and

$$Y_{ij} = p_i b_i a_{ij},$$

giving

$$\frac{X_{ij}}{Z_{ij}} = \frac{t_i b_i a_{ij}}{b_i a_{ij}} = t_i,$$

$$\frac{X_{ij}}{Z_{ik}} = \frac{t_i b_i a_{ij}}{b_i a_{ik}} = t_i \frac{a_{ij}}{a_{ik}}$$

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and similar equations for p_i involving the Y's. We further suppose that in the population of wild chromosomes of which the n actually studied are a sample, the variables t_i, p_i, b_i, a_{ij} are all independent for different subscripts, and are independent when they have the same subscripts, except that t_i and p_i have correlation ρ. The corresponding means and variances are T and σ_t^2, P and σ_p^2, B and σ_b^2, 1 and σ_a^2, where B represents the expected number of Bare-Delta flies per culture, T represents the mean viability of wild homozygotes relative to Bare-Delta, and P the mean viability of Delta/+ heterozygotes relative to Bare-Delta.

The correlation of interest, ρ, between t_i and p_i could be obtained directly from the unknown means, X_{ij}, Y_{ij}, and Z_{ij}, of the observed Poisson variables x_{ij}, y_{ij}, and z_{ij} if they were available. However the observed correlations between

$$w_{ij} = x_{ij}/z_{ij}$$

and

$$v_{ij} = y_{ij}/z_{ij},$$

or between the w_{ij} and v_{ik} will suffer from biases mentioned in the text. In addition, the Poisson variation acts like an "error of observation" causing what is known in psychological statistics as "attenuation", i.e.. a decrease in the numerical value of the correlation by increasing the variances but not the covariances of the variables.

The correction given in the paper was obtained by first finding the expected variances and covariances of the x's, y's and z's, from these finding those of the $1/z$'s, and finally of the w's and v's. These were obtained by series expansion. In the present material, B and n are each of the order of 100. If all terms in the series expansions involving $1/B$ and $1/n$ are ignored, $\sigma_w^2 = \sigma_t^2$, $\sigma_v^2 = \sigma_p^2$, and $\sigma_{w,v} = \sigma_{t,p}$, so that no correction is required. If terms involving $1/B$ and $1/n$ are kept, but those involving $1/B^2$, $1/n^2$, and $1/Bn$ are ignored, the above equalities no longer hold, but for example $\sigma_w^2 = \sigma_t^2 +$ a correction term, or solving, $\sigma_t^2 = \sigma_w^2 -$ the correction term. Using these corrections for the two variances and the covariance, the corrected correlation coefficient can be written as it appears in the text. The algebraic details are too lengthy to include here.

Finally the question of the variance of the corrected correlation arises. Neglecting all but the leading terms of the expansions, the variance of the sample correlation when the population correlation is zero is $1/n$, where n is the number of wild chromosomes tested. When the population $\rho \neq 0$, it must be assumed, as usual, that t_i and p_i are normally distributed. Then the customary large sample formula $\sigma_r^2 = (1 - \rho^2)^2/n$ holds. The tedious algebra needed to take account of the next terms in the expansions involving the Poisson errors and corrections was not undertaken; however, an approximate correction may be obtained as follows. The formula for the corrected correlation is $r_c = rg$, where

$$g = \frac{\bar{z} - 1}{\sqrt{\left(\bar{z} - \frac{\bar{w} + \bar{w}^2}{s_w^2}\right)\left(\bar{z} - \frac{\bar{v} + \bar{v}^2}{s_v^2}\right)}}$$

The condition for g being greater than one is, approximately, that the mean of $\frac{\bar{w}+\bar{w}^2}{s_w^2}$ and $\frac{\bar{v}+\bar{v}^2}{s_v^2}$ should be greater than one. This will surely be true if the coefficients of variation of w_{ij} and v_{ij} are less than one, but this is not necessary. Now the variance of the uncorrected r can be estimated as $(1-r^2)^2/n$, and the variance of $r_c = rg$ will be approximately $g^2(1-r^2)^2/n$. For testing whether r_c is significantly different from zero it is preferable to use the formula $\sigma^2_{r_c} = g^2/n$.

Note that in all these formulas, n is *the number of wild chromosomes tested*, not the number of cultures.

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PROLINE MUTANTS OF SALMONELLA TYPHIMURIUM[1]

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ACCORDING to previous analyses of 16 proline-requiring mutants of *Salmonella typhimurium*, made genetically by NIELSEN (DEMEREC *et al.* 1954) and biochemically by KANAZIR (1956; DEMEREC *et al.* 1955), the mutants fell into at least three groups. Recently, the number of proline-requiring mutants in our collection was increased to more than 200, and useful new techniques were devised or adapted for their study, such as abortive transduction (OZEKI 1956) and recombination between *S. typhimurium* and *Escherichia coli* (BARON, SPILMAN and CAREY 1959; MIYAKE and DEMEREC 1959; MIYAKE 1959; MIYAKE, unpublished). These developments justified further investigations of the proline mutants, which will be reported here.

MATERIALS

The mutants were isolated, by several investigators in M. DEMEREC's laboratory, from the following prototrophic *S. typhimurium* strains: wild type strain LT-2; wild type strain LT-7; and LT-2 (or LT-7) in which a part of the chromosome in the *pro* region had been replaced by a homologous portion from strain LT-7 (or LT-2) through transduction with mutant H4 of phage PLT22. Most of the mutations to proline requirement occurred spontaneously; a few were induced by irradiation with ultraviolet light. Selection was by the standard penicillin screening procedure.

Bacterial mutants are referred to by the symbols proposed in Microbial Genetics Bulletin, No. 16 (1958): *pro* means proline requirement; *glt*, glutamic acid requirement; *cys*, cystine requirement; *lac*$^+$ and *lac*$^-$, ability and inability to utilize lactose.

The bacteriophage in most of the experiments was temperate phage PLT22 H1 (ZINDER and LEDERBERG 1952). Mutants H4 and H5 of the same strain were used to obtain sensitive transductants and to test lysogenicity of bacteria, respectively.

The minimal agar medium for the transduction experiments contained K_2HPO_4, 10.5 gm; KH_2PO_4, 4.5 gm; $(NH_4)_2SO_4$, 1.0 gm; sodium citrate, 0.97 gm; glucose, 2.0 gm; minimal agar, 15.0 gm; demineralized water, 1000 ml. In some experiments the minimal medium was enriched with 0.01 percent dehydrated nutrient broth, to bring about a higher frequency of transduction. When *lac*$^+$

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was the selective marker, sodium citrate was omitted from these media.

The nutrient broth medium in which bacteria were grown or phage stocks prepared contained: NaCl, 4 gm; nutrient broth, 8 gm; demineralized water, 1000 ml. Nutrient agar medium for bioassay of bacteria or phage contained: NaCl, 5 gm; nutrient agar, 23 gm; demineralized water, 1000 ml. EMB agar medium, on which bacteria were tested for sugar fermentation ability, contained tryptone, 10 gm; yeast extract, 1 gm; NaCl, 5 gm; K_2HPO_4, 2 gm; eosin Y, 0.4 gm; methylene blue, 0.065 gm; agar, 15 gm; sugar, 10 gm; demineralized water, 1000 ml.

In supplementing minimal medium with an amino acid, 20 mg was added to 1000 ml of medium.

EXPERIMENTAL RESULTS

Analysis by transduction: For transduction tests, 1 ml of fresh saturated bacterial culture grown in nutrient broth (approximately 2×10^9 bacteria per ml) was mixed with 0.1 ml of phage suspension of T2 buffer containing about 10^{11} phage particles per ml, so as to have a multiplicity (phage/bacterium) of five, and then kept at 37°C for 6–8 minutes while adsorption took place. The mixture was then plated on either minimal or enriched minimal agar, 1/10 ml per plate. Colonies were scored after 24–48 hours of incubation. Large colonies appeared as the result of complete transduction; and minute colonies, which were generally five to 15 times more numerous, resulted from abortive transduction. The fact that the small colonies did originate by abortive transduction was verified by means of OZEKI's (1956) adaptation of NEWCOMBE's respreading technique. The test was made with *proA-15* and wild type phage, and gave positive results. The presence of minute colonies in a transduction experiment involving two phenotypically similar markers is positive evidence that they complement each other, whereas absence of minute colonies indicates noncomplementation (DEMEREC and OZEKI 1959).

The transduction experiments showed that the 127 *pro* mutants tested can be divided on the basis of complementation into four groups: *proA*, *proB*, *proC*, and *proD*. Furthermore, they revealed close linkage between mutants of groups *A* and *B*, since the number of complete transductions was consistently smaller when the experiment involved members of these two groups than when either donor or recipient belonged to another group, or when the donor was wild type. Typical results as to numbers of complete transductants and presence or absence of abortive transductants in inter- and intragroup experiments are given in Table 1. The close linkage between the *proA* and *proB* groups was confirmed by an analysis of multisite mutants, which will be discussed later. Of the 127 mutants tested, 42 are *proA*, 63 *proB*, 18 *proC*, and four *proD*.

Since *proA*, *B*, and *D* mutants can grow on medium containing glutamic-γ-semialdehyde, which does not support growth of *proC* mutants, it was possible to determine whether or not *proC* is carried in the same transducing fragments with other *pro* markers, by transduction experiments in which *proC* was recipient, any of the other mutants was donor, and the selective medium contained glutamic-

TABLE 1

Transduction analysis of proline-requiring mutants of Salmonella typhimurium

Recipients	Donors								
	proA-39	*proA-46*	*proB-9*	*proB-45*	*proC-90*	*proC-95*	*proD-102*	Wild type	Control
proA-39	−	−	+	+	+	+	+	+	−
	0	38	521	105	1,920	2,682	12,342	1,558	0
proA-46	−	−	+	+	+	+	+	+	−
	195	0	269	194	2,746	2,898	14,872	1,678	0
proB-9	+	+	−	−	+	+	+	+	−
	110	19	14	9	1,396	2,040	8,236	868	19
proB-45	+	+	−	−	+	+	+	+	−
	369	60	54	0	2,128	2,440	8,810	1,412	0
proC-90	+	+	+	+	−	−	+	+	−
	1,240	316	1,250	186	0	17	5,388	293	0
proC-95	+	+	+	+	−	−	+	+	−
	545	95	1,122	162	11	0	2,722	279	0
proD-102	+	+	+	+	+	+	−	+	−
	988	96	410	189	526	675	0	359	0

Plus and minus signs indicate presence or absence of abortive transductants. Each figure records number of complete transductants on three enriched minimal agar plates (about 2×10^8 bacteria and 10^9 phage particles per plate). Figures in control column record numbers of spontaneous mutants.

γ-semialdehyde. Colonies of *proA*, *B*, or *D* would appear only when both markers of the experiment were carried in a single fragment. The results, given in Table 2, show that *proA*, *B*, and *C* are located in the same transducing fragment. The *proD* results are negative, but the tests were not conclusive enough to justify a final conclusion.

Biochemical analysis: The pathway of proline biosynthesis has been studied by several investigators in various organisms (see VOGEL 1955). Data available at present indicate that the pathway is as follows:

glutamic acid ⟶ glutamic-γ-semialdehyde ⟶ proline

Therefore the growth responses of *pro* mutants to glutamic acid, glutamic-γ-semi-

TABLE 2

Numbers of wild type and donor type transductants observed in experiments with four proline mutants

Recipient		Donors		
		proA-15	*proB-9*	*proD-102*
proC-90	Wild	2,765	1,838	1,166
	Donor type	3	2	0

Plating on minimal medium supplemented with glutamic-γ-semialdehyde (about 2×10^8 bacteria and 10^9 phage particles per plate).

aldehyde, proline, and hydroxy proline were tested to determine the positions of the blocks for which the mutant genes are responsible. Ornithine was included in the tests because studies by KANAZIR (1956) had indicated that ornithine supports partial growth of one proline group of Salmonella mutants, but all the ornithine tests were negative.

A saturated culture grown in broth was centrifuged, washed, and resuspended in saline, and 0.1 ml of the suspension was plated on minimal agar. A piece of the agar was removed with a sterile cork borer, and 1–2 drops of an aqueous solution of a possible proline precurser (2 mg/ml) was dropped into the hole. After 18–24 hours of incubation, growth responses were examined. The results are summarized in Table 3. They show clearly that in *proA* and *proB* mutants the biosynthesis of proline is blocked somewhere between glutamic acid and glutamic-γ-semialdehyde. They do not differentiate, however, between the two groups. In 16 of the 18 *proC* mutants the requirement cannot be satisfied by glutamic-γ-semialdehyde; therefore, the *proC* block appears to be between glutamic-γ-semialdehyde and proline. The other two (*proC-4* and *proC-124*) show limited growth on hydroxy proline, glutamic acid, and glutamic-γ-semialdehyde. This finding can be explained by the assumption that *proC-4* and *-124* are leaky when grown in medium containing certain intermediate compounds of proline biosynthesis. The nutritional requirement in the four *proD* mutants can be satisfied by either glutamic acid, glutamic-γ-semialdehyde, or proline, so that it appears that the *proD* mutation blocks proline synthesis before glutamic acid. None of these mutants, however, grows as well on medium containing glutamic acid or glutamic-γ-semialdehyde as it does on medium containing proline, and therefore the present determination of the position of the *proD* block should be considered tentative.

Syntrophism among the four complementation groups was studied in the following manner. Bacteria from a saturated broth culture were washed in saline and plated on minimal agar (ca. 2×10^8 cells per plate). A piece of agar was removed, 0.1 ml of a saturated broth culture of another bacterial strain was poured

TABLE 3

Growth responses of proline-requiring mutants to various related chemicals

Loci	Mutants	min*	hp	glt	gs	pro	nut
A	*proA-39*	—	—	—	+++	+++	+++
A	*proA-46*	—	—	—	+++	+++	+++
B	*proB-9*	—	—	—	+++	+++	+++
B	*proB-45*	—	—	—	+++	+++	+++
C	*proC-90*	—	—	—	—	+++	+++
C	*proC-28*	—	—	—	—	+++	+++
C	*proC-4*	—	++	±	++	+++	+++
C	*proC-124*	—	++	++	++	+++	+++
D	*proD-132*	—	—	+	+	++	++
D	*proD-133*	—	—	+	+	++	++

* min = minimal medium; hp = minimal medium supplemented with hydroxy-1-proline; glt = minimal medium supplemented with glutamic acid; gs = minimal medium supplemented with glutamic-γ-semialdehyde; pro = minimal medium supplemented with proline; nut = nutrient medium.

into the hole, and after 18–24 hours of incubation the growth response of the background bacteria was examined. It was found that *proC* feeds either *proA* or *proB*, further evidence for the conclusion that the biosynthetic block effected by mutations in the *proC* locus occurs at a step which is later than the step or steps controlled by *proA* and *B*. No other inter- or intragroup syntrophism was observed.

Multisite mutants: Most of the proline mutants studied revert spontaneously to wild type and recombine with other allelic mutants. They behave as "single-site" mutants, the result of change at a single site of a gene locus. A few, eight out of 206, are "multisite" mutants caused by mutation affecting several, presumably adjacent, sites; they do not undergo reversion. One of these (*proC-110*) covers five out of six sites of the *proC* locus that have been tested, and the other seven extend over various regions of *proA* and *B* (see Figure 1). Three of them (*proAB-47*, *-126*, and *-21*) are associated with sites of both *proA* and *proB*, and provide conclusive evidence that these two are located close together on the chromosome. This evidence, together with the results of the biochemical tests, raises the question whether *proA* and *proB* are two independent gene loci or just two complementation groups of the same locus. The question will be resolved only after the biochemical pathway of proline synthesis becomes better known. In the meantime *A* and *B* will continue to be regarded as two loci.

With the help of overlapping multisite mutants, the relative positions of certain sites of the *pro* loci were determined (Figure 1). The map is very incomplete; a complete map, which would be laborious to prepare, is not needed for the problems considered here.

An unusual and interesting type of behavior was revealed by the analysis of *proAB-47* and *-126*. These two mutants, which originated independently, resemble each other not only in covering all tested sites of loci *proA* and *B* but also in giving rise to much smaller numbers of transductants than other *proA* and *B* or *proC* mutants (Table 4). An interpretation of this behavior is suggested in the discussion.

Analysis by hybridization: It is now well established that crosses can be made between *S. typhimurium* and *E. coli* (Baron *et al.* 1959; Miyake and Demerec 1959; Miyake 1959). Because markers occupying long sections of chromosome

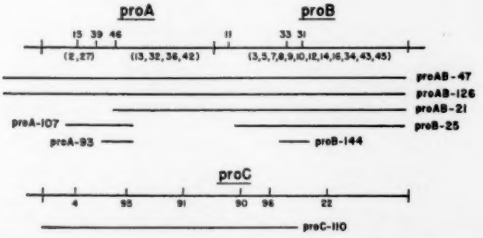


Figure 1.—Diagram indicating the extent of the deletions found in transducing fragments carrying the *proA*, *proB*, and *proC* loci.

TABLE 4

Number of transductions in experiments with two proAB *mutants and with several other* proA *and* proB *mutants*

Recipients	Donors		Control
	Wild type	*proC-95*	
proAB-47	12	26	0
proAB-126	7	9	0
proA-106	960	1,912	0
proA-109	1,088	1,500	0
proB-104	1,020	1,672	2
proB-103	864	1,752	0
proC-90	1,668	57	0
proC-22	1,856	169	5

Figures show number of transductants on four enriched minimal agar plates (about 2×10^8 bacteria and 10^9 phage particles per plate).

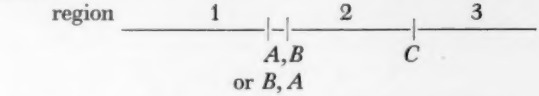
can be recombined in the hybrids, this technique is very effective for genetic mapping of the bacterial genome. It has been used in an attempt to determine the positions of three of the proline loci (*proA*, *B*, and *C*) and *glt*. The last-named locus controls a step in the synthesis of glutamic acid, which is closely related to proline synthesis; *glt* mutants grow on medium containing glutamic acid, but not on medium containing proline. Unfortunately, because the available material is not suitable, the *proD* locus could not be included in these tests.

Since in studies with *E. coli* it had been found that *pro* is located near *lac*, the experiments were made with *S. typhimurium* having the genetic constitution *lac*$^-$ *pro* (or *glt*) and *E. coli* K-12, either Hfr CS-101 (CAVALLI 1950) or Hfr H (HAYES 1953), of the constitution *lac*$^+$ *pro*$^+$ *glt*$^+$. Selection was made for *lac*$^+$, and the hybrids were tested for *pro* or *glt*. About 3000 *lac*$^+$ hybrids were tested for recombination between *lac* and *proA*, about 3000 for recombination between *lac* and *proB*, about 2000 for *lac* and *proC*, and about 300 for *lac* and *glt*. None was found, although recombination took place between *lac* and other markers involved in the tests. Extensive experiments with the hybrids (MIYAKE, in manuscript) indicated that recombination does not occur in the region carrying lactose. The most likely explanation of this finding is that Salmonella, which is *lac*$^-$, is deficient for the *lac* segment of the chromosome, and that recombination in the adjacent regions is reduced when the segment is transferred in hybrids from the *E. coli* to the Salmonella chromosome. Absence of recombination between *lac* and *proA*, *proB*, *proC*, or *glt*, then, indicates that these four gene loci are close together on the chromosome, and suggests that genes controlling related biochemical reactions are grouped together more frequently than can be detected by the transduction method.

DISCUSSION

The data presented show that *proA*, *B*, and *C* are located in the same transducing fragment, because the donor marker is recovered when *proC* serves as recipient and either *proA* or *proB* as donor (Table 2). Transduction frequencies (Table 1) reveal that *proA* and *B* are very close together, with *proC* located some

distance from them. Thus a map of the segment can be represented as shown in the diagram below.



The data of Table 2 show that in the transduction experiment *proC* × *proA* (or *B*) the number of *proA* (or *B*) transductants (recombination in regions 1–3) is very small compared with the number of wild type transductants (recombination in regions 2–3). Since it is reasonable to assume that frequency of recombinations is a function of the length of the regions within which they occur, the results suggest either that region 1 is short or that region 2 is long. If region 1 were short, one would expect considerably fewer wild type transductants in experiment *proA* × wild type, where they originate through recombination involving regions 1 and 2+3, than in experiment *proC* × wild type, where they originate by recombination in regions 1+2 and 3. The data in Table 1 show that the number of wild type recombinants is considerably larger in *proA* (or *proB*) × wild type than in *proC* × wild type, and favor the explanation that region 1 is not short but region 2 is long; they also indicate that region 3 is shorter than either region 1 or region 2.

Further light is thrown on the properties of transducing fragments carrying *pro* loci by the analysis of the two multisite mutants *proAB-47* and *-126*. Each of these mutations covers all the known sites of the *proA* and *proB* loci and behaves like a chromosomal aberration, presumably a deletion. The most interesting feature of the two mutants is that they have much lower frequencies of transduction than other *pro* mutants (Table 4). As mentioned earlier, a small number of transductants is an indication that one or both of the regions in which the responsible recombinations occur are short. The low frequencies thus suggest that the aberrations responsible for *proAB-47* and *-126* extend considerably beyond *proA* and *B*, making either one or both of the regions between the ends of the aberration and the corresponding ends of the transducing fragment very short. We can deduce, however, that only the end distant from the *proC* locus is involved, from the fact that the aberrations do not affect *proC*. This deduction is further confirmed by the results of experiments *proAB-47* (or *-126*) × wild type and × *proC-95* (Table 4), in which similar numbers of transductants were obtained with wild type and with *proC* donors. Thus the analysis indicates that mutants *proAB-47* and *-126* are due to chromosomal aberrations that begin approximately at the end of locus *proA* or *proB*, whichever is nearer to *proC*, and extend nearly to the end of the transducing fragment farthest from *proC*. Recombinations do not take place within the aberrant region. It is interesting to note that the phenotypes resulting from these two aberrations do not differ from those of single-site *proA* or *B* mutants. If the aberrations are deletions, this fact suggests that the deleted region outside *proA* and *B* either carries genes that produce no detectable effects or carries no genes at all, is genetically inert.

Among the numerous multisite mutants that have been analyzed in Salmonella only *proAB-47* and *-126* have lower frequencies of transduction than single-site

mutants of the same locus. It seems unlikely that the identification of two such rare mutations in the same region of the chromosome is due to chance. More probably, such changes occur with a higher than average frequency in this particular region. A comparable phenomenon has been observed in the *cysC* and *D* loci, where about 40 percent of the mutations belong to the otherwise rare category of multisite mutants (DEMEREC 1956).

SUMMARY

By means of genetical and biochemical tests, 127 proline-requiring mutants of *Salmonella typhimurium* were classified in four groups: 42 in *proA*, 63 in *proB*, 18 in *proC*, and four in *proD*. In the sequence of proline biosynthesis *proC* mutants are blocked between glutamic-γ-semialdehyde and proline, *proA* and *B* mutants before glutamic-γ-semialdehyde, and *proD* mutants apparently before glutamic acid. The results of transduction tests show that the *proA*, *B*, and *C* loci are carried by the same transducing fragment; *proA* and *B* are close together and *proC* is some distance from them. Hybridization experiments with *Escherichia coli* revealed that *proA*, *proB*, *proC*, and *glt* are located close to the *lac* locus.

Eight of the mutants are due to multisite mutations, presumably deletions, of which two (*proAB-47* and *-126*) cover both the *proA* and *proB* loci and extend a considerable distance toward one end of the transducing fragment. If these mutations are deletions, then the deleted region beyond *proA* and *B* either carries no genes—is genetically inert—or carries genes that produce no detectable effects.

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THE MUTATIONAL BEHAVIOR OF THE R^r:JANA ALLELE IN ZEA MAYS[1]

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PREVIOUS studies (STADLER and NUFFER 1953; STADLER and EMMERLING 1954) have shown that the action of the standard R^r allele upon anthocyanin is due to two closely linked genes which are separable by crossing over. The identification of a sufficient number of crossovers within the R complex, together with evidence on the sequence of mutational steps, proved the existence of independent components.

However, most of the experimental evidence on which this conclusion is founded is derived from studies with the allele R^r:Cornell. STADLER (1946), working with various R^r alleles of diverse origin, found that spontaneous mutation usually affects seed color and plant color independently. But in none of the former studies had there been a check on crossing over. Consequently, it remains to be determined whether all R^r alleles possess separable components.

The most common type of mutational sequence observed by STADLER (1948) is illustrated in Figure 1.

The R^r alleles which mutate regularly to r^r (seed color mutation) and R^g (plant color mutation) are Cornell, Boone, Quapaw, Ponca, and Black. Both common types of mutants, r^r and R^g, have given mutations to r^g (loss of seed color and plant color effect). In this group of R^r alleles, mutations of R^r to r^g were rarely found, indicating that the action of the R segment is due to two separate genes rather than to the action of a single gene.

However, this same investigation revealed that the stepwise course of spontaneous mutation shown by R^r:Cornell, Ponca, etc., is not characteristic of all R^r alleles. STADLER (1948) reported that two R^r alleles with dilute pigmentation (R^r:Catspaw and Winnebago) mutate directly to r^g and not to r^r. In the case of these two alleles, he concluded that both plant and seed color are dependent upon a single genetic component.

The present report is concerned with a third type of R^r allele which mutates regularly to r^g and less frequently to r^r. In contrast to R^r:Catspaw and Winne-

FIGURE 1.—Mutational sequence of R^r:Cornell, Boone, Quapaw, Ponca, and Black.

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bago, this allele which is known as R^r:Jana is identified by strong plant color both in the seedling and the flowering stages.

The first indication of a striking difference between R^r:Jana and R^r:Cornell was the finding of a r^g mutant in the heterozygote R^r:Jana/R^r:Cornell. Since this type of mutant was not expected from mutation of R^r:Cornell, it was suspected that it originated from R^r:Jana. Consequently, a more extensive analysis was made of the types of seed color mutants occurring in stocks homozygous for R^r:Jana. The present study gives no indication of the frequency of mutations affecting plant color.

MATERIAL AND RESULTS

The seed mutation data from the cross of ♀♀ *g* R^r:Jana *K*/*G* R^r:Jana *k* × ♂♂ *g* r^g *k*/*g* r^g *k* are summarized in Table 1. The stocks of homozygous R^r:Jana were marked on either side of the *R* complex with *g* (golden plant; 14 units from *R*) and *K* (abnormal knob 10; one unit from *R*).

Out of a total population of 245,515 female gametes tested, 24 colorless seed mutants were analyzed, and of these all but two were r^g; none of these cases exhibited defective pollen. Of the 22 r^g mutants produced, 13 were *g* r^g *K*, seven were *G* r^g *k*, and two were *G* r^g *K* in constitution. The simplest explanation of the origin of these cases is that the plant and seed color determiners of R^r:Jana mutated simultaneously to the double recessive or to r^g. On this assumption the *g* r^g *K* mutants would be attributed to mutations in the *g* R^r *K* chromosome, and the *G* r^g *k* mutants to mutations in the *G* R^r *k* chromosome. The origin of the two *G* r^g *K* cases in which a crossover occurred may be ascribed to mutations in the *g* R^r *K* chromosome with a coincidental crossover between *g* and *R*, since the number of crossovers expected by coincidence is about three (or 14 percent). The possibility that these two cases arose from mutations in the *G* R^r *k* chromosome with a simultaneous crossover between *R* and *K* is unlikely because of the low frequency of crossing over in this region (one percent).

It will be seen in Table 1 that homozygous R^r:Jana also produced two seed

TABLE 1

The frequencies and types of seed color mutants observed in progenies from homozygous R^r:*Jana.* ♀♀ gR^rK/GR^rk × ♂♂ gr^gk/gr^gk

Culture	Number of seeds examined	Number of colorless seeds	Mutant fraction determined	Phenotype of mutant individuals			
				gr^rk	gr^gk	Gr^gK	Gr^gk
56:825	75,525	6	4/6	1	3	0	0
826	66,970	9	7/9	0	4	0	3
827	23,460	5	3/5	0	2	1	0
828	26,480	1	1/1	0	0	0	1
829	31,135	6	5/6	1	2	1	1
830	21,200	3	3/3	0	1	0	2
58F 3245	745	1	1/1	0	1	0	0
Total	245,515*	31	24/31	2	13	2	7

* Adjusted mutation frequency 24/31 × 245,515 = 24/190,076.

color mutants of type $g\ r^r\ K$. These are mutants that would be expected if the plant and seed color effects of the R^r segment were due to independent components. On this basis the origin of the two $g\ r^r\ K$ mutants would be ascribed to mutations of the seed color determiner R to r in the $g\ R^r\ K$ chromosome since the mutant chromosomes are of the same g and K constitution as the $g\ R^r\ K$ chromosome.

The most striking demonstration of the difference in mutability comes from a comparison of the types of seed color mutants occurring in stocks homozygous for R^r:Jana and R^r:Cornell. The mutation data of R^r:Cornell along with the data of Table 1 are shown in Table 2. It is evident from this comparison that the r^g class of mutation does not occur in the progeny of R^r:Cornell in spite of the large numbers tested. Of the 110 mutants analyzed in the progeny of R^r:Cornell, approximately 44 percent were the result of oblique crossing over, and 56 percent were the result of intragenic alterations, designated as noncrossover mutations. In the case of homozygous R^r:Jana, all of the mutants tested were mutations of the noncrossover type with the possible exception of the two $G\ r^g\ K$ mutants. As mentioned previously, these two cases could be due to noncrossover mutation with a coincidental crossover in the G–R segment.

It is also apparent from the data of Table 2 that the total mutation rate is higher in R^r:Cornell than in R^r:Jana. The calculated frequency of mutation for R^r:Cornell is 3.17×10^{-4}, while the rate determined for R^r:Jana is only 1.26×10^{-4}. This difference could be explained as being due to modifying genes since the data were obtained from different stocks and also in separate growing seasons.

In general it is evident that R^r:Jana mutated more frequently to r^g (rate $= 1.16 \times 10^{-4}$) than to r^r (rate $= 0.11 \times 10^{-4}$), and that the mutations were chiefly the result of noncrossover alterations.

A further study was made of R^r:Jana in compounds with R^g noncrossover-1, a plant color mutant derived from R^r:Cornell. In a previous publication (EMMERLING 1958), it was reported that the R segment of R^g noncrossover-1 possesses a recessive plant color component (designated p) and a dominant seed color component (designated S), and that p and S are synaptically homologous. This resulted in plants heterozygous for p S K and PS:Cornell k, in the production of two classes of seed color crossovers, as illustrated in Figure 2. The occurrence of oblique crossing over produced the apparent mutants $g\ p^{(s)}k$ (or $g\ r^g\ k$) and $G\ P^{(s)}K$ (or $G\ r^r\ K$). In addition to the crossover mutants, the compound g p S K/G P S:Cornell k yielded two classes of noncrossover mutants namely, g p s K

TABLE 2

Comparison of the types and frequencies of aleurone color mutants from homozygous R^r:*Cornell and* R^r:*Jana*

Parent	Population	Mutants	Rate (10^{-4})	Noncrossovers		Crossovers		Exceptional cases		
				gr^rK	Gr^rk	Gr^rK	gr^rk	gr^gK	Gr^gK	Gr^gk
$g\ R^r$:Cornell $K/G\ R^r$:Cornell k	346,794	110	3.17	28	34	25	23	0	0	0
$g\ R^r$:Jana $K/G\ R^r$:Jana k	190,076	24	1.26	2	0	0	0	13	2	7

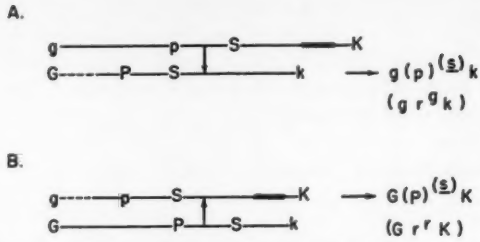

FIGURE 2.—Oblique crossover mutants resulting in loss of seed color.

(or *g* r^g *K*) resulting from mutation in the *g* P S *K* chromosome, and *G* P s *k* (or *G* r^r *k*) resulting from mutation in the *G* P S *k* chromosome.

Thus if R^r:Jana were like R^r:Cornell in its mutational behavior, the heterozygote R^r:Jana *k*/R^g noncrossover–1 *K*, should yield noncrossover mutants of types r^r *k* and r^g *K*, and also oblique crossover mutants of types r^r *K* and r^g *k*. But in view of the results reported herein, the data from the heterozygote are complicated by the fact the R^r:Jana mutates to r^g in one step. In other words, the origin of the r^g *k* mutant could be ascribed either to mutation in the R^r:Jana *k* chromosome, or to the occurrence of oblique crossing over (Figure 2, type A). However, the information obtained from tests of the heterozygote does provide a basis for estimating the frequency of r^r mutations from R^r:Jana, and for determining whether the plant and seed color effects of R^r:Jana are separable by crossing over.

Two stocks of R^r:Jana/R^g noncrossover–1 were used as female parents in backcrosses to r^g/r^g (Table 3). Section A includes the results from the cross *G* R^r:Jana *k*/*g* R^g noncrossover–1 *K* × *g* r^g *k*/*g* r^g *k*, in which the stock carried both *g* and *K*, and section B similarly includes tests of R^r:Jana *k*/R^g noncrossover–1 *K* but without the *g* marker.

In culture A, 20 mutants were analyzed, and of these 18 were r^g and two were r^r. Of the 18 r^g mutants produced, eight were of type *g* r^g *K* resulting from mutations in the *g* R^g *K* chromosome, and one was of type *G* r^g *K* originating from mutation in the *g* R^g *K* chromosome with a coincidental crossover in the *g*—*R* region. The other nine r^g cases are of the type which could be produced by mutation of R^r:Jana to r^g as well as by oblique crossing over. However, the most probable mechanism involved in the production of six of the nine mutants, the *G* r^g *k* cases, is that they arose from gene mutations in the *G* R^r:Jana *k* chromosome to *G* r^g *k*. If these six cases were ascribed to the occurrence of oblique crossing over (Figure 2, type A), it would be necessary to assume that a crossover occurred between P and S as well as a crossover between *G* and P. The expected frequency of this double crossover is approximately 0.005 percent. In the case of the three remaining mutants of type *g* r^g *k*, oblique crossing over could account for the phenotype observed without postulating the occurrence of a second crossover in the *g*—*R* segment. Another possibility to consider in regard to the origin of the three *g* r^g *k* cases is that they arose from gene mutations in the *G* R^r:Jana *k* chromosome with a coincidental crossover in the *g*—*R* region.

TABLE 3

The frequencies and types of seed color mutants observed in progenies from heterozygous R^r:*Jana.* ♀♀ R^r *Jana*/R^g *Cornell* × ♂♂ r^g/r^g

Culture	Number of seeds examined	Number of colorless seeds	Mutant fraction determined	Phenotype of mutant individuals								
				$r^r k$		$r^g K$		$r^r K$		$r^g k$		Df‡
A. GR^r:Jana k/gR^g:Cornell K				*g*	*G*	*g*	*G*	*g*	*G*	*g*	*G*	
56: 822	47,665	12	11/12	0	0	5	0	0	1	2	3	0
75:1308	8,137	4	4/4	1	0	1	1	0	0	0	1	0
1309	7,262	2	0/2	0	0	0	0	0	0	0	0	0
1317B	2,157	1	1/1	0	0	1	0	0	0	0	0	0
1415	3,354	1	0/1*	0	0	0	0	0	0	0	0	0
77:1563	10,897	3	3/3	0	0	1	0	0	0	0	2	1
1564A	3,249	1	1/1	0	0	0	0	0	0	1	0	0
1564B	2,405	0	0/0	0	0	0	0	0	0	0	0	0
Total	85,126	24	20/24	1	0	8	1	0	1	3	6	1
B. R^r:Jana k/R^g:Cornell K												
56: 820	48,628	4	4/4	0		3		0		1		1
821	80,243	20	17/20	0		12		0		5		1
823	62,590	13	13/13	1		6		1		5		2
824	25,539	8	6/8	0		5		0		1		0
75:1318	2,855	1	1/1	0		0		0		1		0
Total	219,855	46	41/46	1		26		1		13		4
Sum total	304,981†	70	61/70	2		35		2		22		5

* One mutant not analyzed.
† Adjusted frequency 61/265,769.
‡ Number of visible cytological deficiencies.

The origin of the one *G* r^r *K* mutant may be attributed to two alternative mechanisms. The most probable one is that it originated as the result of oblique crossing over (Figure 2, type B). Another less likely possibility is that it arose by mutation in the *G* R^r:Jana *k* chromosome to *G* r^r *k* followed by a crossover in the *R*—*K* region. However, this event is expected only in one percent of the mutants analyzed. In the case of the one mutant of type *g* r^r *k*, the most probable mechanism that could account for its origin is by gene mutation of *G* R^r:Jana *k* to *G* r^r *k* with a simultaneous crossover in the *g*—*R* segment.

In culture B, 41 mutants were analyzed, and of these one was of the r^r *K* class resulting from either oblique crossing over or mutation with a coincidental crossover in the *R*—*K* segment (one percent), and one was of type r^r *k* originating from mutation of R^r:Jana *k* to r^r. Of the 39 r^g mutants produced, 26 were r^g *K* in constitution and 13 were r^g *k* in type. The r^g *K* cases may be ascribed to mutations of the R^g *K* chromosome, while the r^g *k* mutants may be attributed to either mutations of R^r:Jana to r^g or to the occurrence of oblique crossing over (Figure 2, type A).

The totalled data for cultures A and B in Table 3 show that 57 of the 61 mutants analyzed were r^g in constitution, and of these, 22 could be ascribed to mutations

of R^r:Jana if oblique crossing over of type A were excluded. The total mutation rate of R^r:Jana to r^r was 0.08×10^{-4} (2/265,769). The frequency of oblique crossing over was 2/265,769 (0.08×10^{-4}). In addition to mutations, five plants were found to have cytological deficiencies and abortive pollen.

CONCLUSIONS

It seems clear from the comparison of the homozygous R^r:Jana and homozygous R^r:Cornell that a large proportion of the seed color mutants from R^r:Jana were of the type that would be expected if (P) and (S) were a single entity with two kinds of action. Whether, however, the R segment of R^r:Jana actually consists of a single element cannot be definitely concluded on the basis of the present results since a small proportion of the mutants were of the type expected from the action of two independent genes. For the moment, the significance of this finding still remains unclear. Although it is possible that the seed color factor of R^r:Jana mutated independently of the plant color element, it is also conceivable that the origin of the r^r mutants may be attributed to some other mechanism which is not apparent at the present time. It is also puzzling, in view of the high frequency of oblique crossing over in stocks of R^r:Cornell, to find so few oblique crossovers in the progeny of R^r:Jana. It may be that the structure of R^r:Jana is a complex of two spatially segregated parts so oriented as to be nearly incapable of separation by crossing over. Furthermore, the available data on Jana give no evidence of the frequency of mutations involving loss of the plant color effect (R^g) without loss of the seed color effect. To test this possibility it would be essential to examine a large number of seedlings from homozygous R^r:Jana in order to identify the plant color mutants. Consequently, it would be decidedly premature to conclude from the evidence on hand that the action of R^r:Jana is due to a single element.

One may suggest from these results that the origin of the r^g mutants is due to a suppressive type of mechanism, inhibiting both plant and seed color. However, there is no evidence that indicates such a mutator system is present in the stocks of R^r:Jana. Approximately 200 seeds of each of the r^g mutants were closely examined for dominant mutations, but no spots or sectors of colored aleurone were found. A similar examination was made of the plant tissues, both in the seedling and flowering stages and also in the seedling stage of the F_1 progeny. Again no evidence of reverse mutations was found. It might be relevant to mention here that another R^r allele was found whose mutational behavior is similar to R^r:Jana, but its mutant progeny were highly unstable. This allele, which is known as R^r:Nanson, mutates directly to r^g in one mutational step and also to R^g. However, in succeeding generations reverse mutations were identified in the aleurone and plant tissues.

Another way of explaining the observed r^g mutants is on the assumption that in R^r:Jana, null elements are present either to the left or right of the R^r segment. This would result in the production of gametes with allelic types equivalent to r^g if crossing over occurred following oblique synapsis of the null element with either (P) or (S) (see STADLER and EMMERLING 1956 for discussion of null elements). However, if oblique crossing over were the sole cause of the r^g mutations,

all of the mutants should show the occurrence of crossing over in this interval but not in the *G—R* segment except for the possible occurrence of double crossing over, which is improbable within this short segment. To account for the origin of the r^g mutants observed in terms of null elements, the occurrence of double crossing over would have to be assumed in the parental stocks of 20 of the 22 r^g mutants identified. Thus, it does not seem likely that oblique crossing over was the major source of the exceptional r^g mutants.

SUMMARY

The allele R^r:Jana mutates regularly to r^g (colorless seed, colorless plant) and less frequently to r^r (colorless seed, colored plant). These results were compared with those of a previous experiment involving the allele R^r:Cornell, in which the R^r segment mutates with appreciable frequency to r^r and rarely to r^g. The pronounced difference between the two R^r alleles may be ascribed to the apparent absence of two separable seed and plant color components in the case of R^r:Jana, and the existence of two independent elements within the *R* segment of R^r:Cornell. Alternative explanations were discussed.

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THE EFFECT OF PATERNAL X-RAY EXPOSURE ON THE SECONDARY SEX RATIO IN MICE (F_1 GENERATION)[1]

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WHEN the mouse testis receives an X-ray dose greater than 350r, fertility ceases within four weeks and, after an interval of sterility which may last several months (depending on the dose), fertility is restored. During the presterile and poststerile periods, matings with unirradiated females yield significantly different litters. RUSSELL (1954) has reviewed the earlier work on this point, especially the important experiments of SNELL (1933), HERTWIG (1938), and STRANDSKOV (1932), as well as his own. Litter size in the presterile period decreases by 15 to 85 percent as the X-ray dose is increased from 200 to 1000r, but the sex ratio of the progeny remains constant. The reduction in litter size is the result of embryonic or fetal death, not of a failure in fertilization. These results are analogous to those in Drosophila, with the exception that in the latter the sex ratio tends to change in favor of the males (CATCHESIDE and LEA 1945).

In contrast, the change in litter size during the poststerile period is small or questionable, as is the change in sex ratio, even after doses up to 1200r. The difference is considered to be due to the type of germ cell irradiated: the spermatozoa of the presterile period were irradiated as such, or matured from irradiated spermatids, or perhaps spermatocytes; the spermatozoa of the poststerile period are derived from irradiated stem cells whose progeny matured successfully.

In the course of an experiment designed for another purpose, in which irradiated males were mated in the poststerile period with unirradiated females, we observed an increase in the sex ratio of the progeny from 42 to 52 percent males, which was significant at the five percent level by the χ^2 test and obviously differed from results reported in the literature. In view of the exceptionally low sex ratio in our unirradiated stock (42 percent males), we entertained the possibility that this stock might be peculiarly suitable for the demonstration of such an effect. In order to investigate the matter further, the original experiment was enlarged and a second independent one was set up in which the regression of sex ratio on X-ray dose was determined. The data from both experiments are reported here.

MATERIALS AND METHODS

The mice used in these experiments were obtained from the Cancer Research Genetics Laboratory, University of California, Berkeley. The CAF_1 males were

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produced by mating BALB/cCrgl females with A/Crgl males. The original BALB/c stock, in its 50th generation, was obtained from Andervont (National Cancer Institute) and was bred through 11 more generations; the original A/He stock, in its 84th generation, was obtained from Heston (National Cancer Institute) and was bred through 15 more generations.

The animals used in our experiments were fed Purina Laboratory Chow Checkers and water *ad lib.*

The CAF_1 males were irradiated to the entire body (Experiment 1) or to the posterior 2.5 cm of the body (Experiment 2), as described previously (KOHN and KALLMAN 1956). The radiation factors were: 250 kvcp X-rays, HVL 1.5 mm Cu; exposure dose rate in the testes, 40–44r/min. The absorbed dose (rads) in the testes was taken to be five percent less than the exposure dose (r) in the testes.

The matings were carried out in both of the experiments by placing one male in a cage with three or five females for four or five weeks. When a female became pregnant, she was placed in a separate cage in which she raised her litter until weaning (at about age 35 days). In the first experiment the cages were examined only five days a week so that the data at birth were less accurate than those of the second experiment, in which the cages were examined at least once each day, and the progeny were sexed both at birth and at weaning. The data at weaning were used to correct errors in sexing at birth; about one percent of the diagnoses at birth required this correction.

RESULTS

Experiment 1: The CAF_1 males that had been irradiated (whole body) at age 385 days (±30 days) were mated at age 700 days (±30 days) with virgin unirradiated BALB/c females (ages 71–113 days).

It was considered important that the general health of the samples of males selected for breeding should be uniform. Therefore, the 18 males mated from each dose group were selected at random from the members of the group weighing at least 26 gm and having lost less than 2 gm during the preceding three months.

In brief, for the three dose groups, the sex ratio (fraction of males) at weaning was as follows:

(1) 0 rads: 216 progeny, sex ratio, 0.416;

(2) 525 rads: 182 progeny, sex ratio, 0.522;

(3) 2.× 262 rads: 239 progeny, sex ratio, 0.397.

The low sex ratio of the control group, 0.42, was not unexpected, since ratios in the range 0.44–0.48 were being obtained in BALB/c and CAF_1 matings; it was therefore not considered primarily due to the advanced age of the fathers. The sex ratio of the group given two doses of 262 rads (eight days apart) did not differ significantly from that of the controls. The high sex ratio for the group given 525 rads was surprising. Statistical tests (χ^2) showed significant heterogeneity among the three groups and that the result at 525 rads was significant at the five percent level.

To examine the matter further, it was decided to mate the males again (the

median life span of the CAF_1 male is 870 days). Of the 18 males originally in each group, the following survived for the second mating (at about 820 days of age): 16 in group 1; ten in group 2; 16 in group 3. Each male was caged for five weeks with five females (one of whom had previously had a litter). The combined results for both matings are shown in Table 1. The elevation in sex ratio of group 2 was no longer significant, and the result for the pooled data of groups 2 and 3 (the irradiated groups) was almost identical to that of the control group. It was concluded that the difference in sex ratio originally observed may have been the result of random variation and that any further decision would have to rest on the results of a second independent experiment. With respect to progeny per litter, irradiation appeared to have reduced this from 5.5 to 5.0.

Experiment 2: Nine-month-old CAF_1 males (ten per group) were irradiated to the pelvic region; the absorbed doses received by each group are detailed in Table 2. Seven months later a series of four breedings five weeks apart was begun, arranged so that the first two were with virgin females (age 75–150 days) and would yield first litters, and the third and fourth would yield principally second litters. From each dose group, seven males were used; the ratio was three females per male: a total of 672 litters for eight groups was possible in four breedings—the yield was 577, or 86 percent.

The first breeding employed 168 virgin BALB/cCrgl females. The second breeding employed another 168 virgin females. The third breeding employed 116 females from the first breeding (whose litters were weaned) plus 52 virgins. The fourth breeding employed 132 females from the second breeding plus 36 virgins. Each male participating in the four breedings, therefore, had been caged with a total of 12 females; in each breeding the distribution of females was strictly random. One breeding male died in each of groups 6, 7, and 8, and was replaced by a "spare". Also, in groups 4, 5, and 8, one breeding male from each whose fecundity seemed low was replaced by a spare.

The over-all experimental design allowed two subsidiary factors to be examined for their possible influence on sex ratio and litter size.

First, the results showed that at birth and at weaning the sex ratio is practically the same and that it is independent of the parity of the female (zero or one), although the size of the first litter is smaller than the second, as is well known.

TABLE 1

Experiment 1: Number and sex ratio of progeny at weaning from mating whole-body irradiated CAF_1 males with unirradiated BALB/cCrgl females

Group	Dose (rads)	Litters (at birth)	Males	Females	Total: ♀+♂	Sex ratio*	Progeny/ litter†
1	0	81	185	260	445	0.416	5.5
2	525	63	145	168	313	0.46	5.0
3	262 × 2	84	161	268	429	0.38	5.1
Pooled:							
2 + 3		147	306	436	742	0.413	5.0

* ♂/(♂ + ♀).
† (Total weaned)/(No. of litters).

TABLE 2

Experiment 2: Number and sex ratio of progeny at birth from mating partial-body irradiated CAF_1 males with unirradiated BALB/cCrgl females

Breeding*	Dose group (rads)	Litters	Male	Female	Sex ratio	♂/Litter†	♀/Litter†	Progeny/litter†,‡
1–4	All	577	1,709	2,181	0.439	2.96	3.78	6.81
1 + 2	All	279	752	955	0.441	2.70	3.42	6.17
3 + 4	All	298	957	1,226	0.438	3.21	4.11	7.41
1 + 3	All	297	832	1,113	0.428	2.80	3.75	6.59
2 + 4	All	280	877	1,068	0.451	3.13	3.81	7.04
1–4	0	76	234	287	0.449	3.08 ± 1.7	3.78 ± 1.8	6.95 ± 2.2
	248	75	205	304	0.403	2.73 ± 1.6	4.05 ± 1.8	6.80 ± 2.4
	360	78	234	274	0.461	3.00 ± 1.8	3.51 ± 1.9	6.68 ± 2.6
	495	72	232	266	0.466	3.22 ± 1.6	3.69 ± 1.9	6.96 ± 2.4
	248 × 2	74	246	288	0.461	3.32 ± 1.6	3.89 ± 1.8	7.32 ± 2.1
	610	70	211	263	0.445	3.01 ± 1.7	3.76 ± 1.8	6.77 ± 2.5
	720	66	182	264	0.408	2.76 ± 1.6	4.00 ± 2.0	6.85 ± 2.5
	248 × 3	66	165	235	0.412	2.50 ± 1.7	3.56 ± 1.6	6.08 ± 2.5

* In breedings 1 and 2, the females were virgin; in breedings 3 and 4, 75 percent had had one litter. In breedings 1 and 3 the females were from one pool, and in breedings 2 and 4, from another one.
† ± standard deviation.
‡ Thirty-nine partially eaten progeny that could not be sexed are included in progeny per litter.

This conclusion is based on comparison of the pooled data of breedings 1 and 2 (first litters only) with those of breedings 3 and 4 (over 70 percent second litters). The sex ratio was 0.44 in both groupings at birth, but the mean litter sizes were 6.2 and 7.4, respectively (Table 2). At weaning, the corresponding figures were 0.45, 4.8, and 5.4.

Secondly, although the sex ratio may have depended slightly on variations between different populations of females, the indicated effect was quite small and was not statistically significant. The sex ratio at birth for breedings 1 and 3 was 0.43, for breedings 2 and 4, 0.45; similar results were obtained at weaning (Table 2).

In Experiment 1, the preliminary result (but not the final one) had suggested that fractionating the X-ray dose might influence the ratio. In Experiment 2, fractionation of the dose did not affect the sex ratio at birth or weaning, nor did it have a consistent effect on litter size (dose groups 248 × 2 and 248 × 3 compared with 495 and 720, respectively (Table 2).

To estimate the effect of dose, unweighted linear regressions of the form $y = a + bx$ were calculated, in which y is the mean number of males, of females, or of males plus females per litter (or, the sex ratio), and x is the absorbed dose in rads. The values for the parameters a and b are given in Table 3. In no instance was the slope b significantly different from zero.

Since Experiments 1 and 2 were quite independent, the sex ratios at weaning may be compared. As noted above, the single-dose and fractionated dose groups of the first experiment did not differ from the control, nor did the corresponding groups in the second experiment (495 and 248 × 2). These corresponding groups

TABLE 3

*Values of parameters in regression lines**

y	a	b
At birth†		
Male/litter	3.123	−0.000372±0.000417
Female/litter	3.819	−0.000086±0.000314
(Male + female)/litter	7.040	−0.000520±0.000528
Male/(male + female)	0.4509	−0.000028±0.000041
At weaning		
Male/litter	2.489	−0.000376±0.000237
Female/litter	2.791	+0.000031±0.000309
(Male + female)/litter	5.281	−0.000351±0.000321
Male/(male + female)	0.473	−0.000045±0.000043

* $y = a + bx$, where x is the absorbed dose in rads.
† Based on data in Table 2.

within each of the two experiments were therefore pooled, with the following results:

Experiment 1: 491 males, 696 females; sex ratio 0.414

Experiment 2: 545 males, 602 females; sex ratio 0.475

The value of χ^2 is 8.94, and $P < 0.005$. The sex ratio was therefore significantly different in the two experiments.

DISCUSSION

Two sets of experimental data in the literature for large numbers of mice from irradiated fathers are available (HERTWIG 1938, RUSSELL 1954). The chief conclusion from these and our own, drawn with due regard to the possibility of inter- as well as intraexperimental variation, is that for exposures up to 1000r a clear-cut change in sex ratio has not been demonstrated. If a change is indicated, it is probably in the direction of a decrease. The specific results are as follows:

HERTWIG pooled the data from various experiments in her laboratory, chiefly with the dilute blue strain (*dd bb aa CC*). She concluded that no significant change in sex ratio occurred after exposures of 400–1000r. We have fitted an unweighted regression line (sex ratio $= a + bx$) to these data based on 2595 control progeny and 2006 progeny from irradiated fathers; $a = 0.4931$, $b = +0.0000177 \pm 0.0000204$. After 1200–1600r, the ratio rose abruptly to about 0.59. HERTWIG remarked that this last result, based on only 234 progeny, depended on the abnormally high ratios associated with several fathers, rather than a uniform increase in the ratio for all, and that further study was needed.

RUSSELL, for the stock used in his genetics experiments, found a sex ratio of 0.5100 (55,828 progeny) in the controls and 0.5035 (72,472 progeny) after 600r. Calculated by us, the slope b, based on these two points, is -0.000011. The difference between the controls and experimental animals is barely significant: $\chi^2 = 5.3$; $P \backsim 0.025$.

In the second experiment of the present series, the regression analysis, based on 3890 progeny at birth, estimated that $a = 0.4509$ and $b = -0.0000279 \pm$

0.0000412 (Table 3). In the first experiment, based on two points (zero and 525 rads) with 1187 progeny (at weaning), $a = 0.416$ and $b = -0.000006$.

The results with mice are at variance with the conclusions of SCHULL and NEEL (1958) for man, based on their study of Japanese data. For the comparable class of Japanese data (exposure of fathers only), there were the following estimated exposures and progeny: 0r, 46,166; 8r, 5463; 75–100r, 1309; 200r, 753. The value of b was + 0.000056, but it was not significantly different from zero. SCHULL and NEEL, however, concluded that a significant increase had occurred, based on the following argument: they predicted the changes in the mammalian sex ratio after irradiation on the assumption that the Y chromosome is physiologically inert, whereas the X chromosome is susceptible to injury that can express itself as a dominant lethal; they found that the signs of the slopes of the regression lines fitted to several classes of data were in accord with the theory, even though the slopes of the individual regression lines were not significantly different from zero.

To the writer, such an argument seems insecure. SCHULL and NEEL were, of course, aware of the fact that small changes in the sex ratio might stem from causes other than radiation (Brit. Med. Journal Editorial 1953; MACMAHON and PUGH 1954). Furthermore, it is now known that the Y chromosome is physiologically active, exerting a positive masculinizing force in the mouse (WELSHONS and RUSSELL 1959) and in man (JACOBS and STRONG 1959; FORD, POLANI, BRIGGS and BISHOP 1959). The specifically stated theoretical basis of SCHULL and NEEL's argument has therefore been proved incorrect. The argument, however, might be advanced in a modified form, namely, that the smaller size of the Y chromosome renders it less likely to be the site of a dominant lethal hit than the X chromosome. Only further experimentation can provide the proof for this conjecture. Meanwhile, it is suggested that our view of the effect of radiation on the sex ratio in mammals be an empirical one.

In the present experiments, X-rays had no effect on the sex ratio as tested by linear regression (Table 3). The validity of the linear hypothesis, however, might be questioned. The sequence of ratios in Table 2 suggests but does not establish that the sex ratio fell after 248 rads, then returned to the normal range, and finally fell once more after 720 rads. It is of interest that the results of duplicate, independent determinations at 495 and 720 rads appeared to be in much closer agreement than would have been expected if the observed variation between doses had been a good estimate of random variation. An indication of a nonlinear response has been observed in the case of X-ray treated rooster sperm; after about 250r, the sex ratio fell, but it was normal after higher doses (DEMPSTER, LERNER and INOUYE 1959).

A significant change in sex ratio occurred between the first (0.414) and second (0.475) experiments of the present series; the cause is not known, but the difference in paternal ages may have been a contributing factor. The difference in radiation exposure (partial- and whole-body) is considered to be an extremely unlikely factor. In this connection, it may be noted that although genetic factors are known to be important determinants of the variations in sex ratio (KING 1918), the mechanisms involved have not been precisely described in mice

(HOWARD, MCLAREN, MICHIE, and SANDER 1955; WEIR, HAUBENSTOCK and BECK 1958), nor have generally accepted theories been found to cover the differences between or within other mammalian species (GINI 1951; DAHLBERG 1951; MCKEOWN 1956–57; LINDAHL and SUNDELL 1958).

SUMMARY

CAF_1 male mice, irradiated with 250 kv X-rays, were mated during the post-sterile period with unirradiated BALB/cCrgl females. For absorbed doses in the range 0–720 rads, the slopes of the following regression lines were not significantly different from zero: males per litter, females per litter, progeny per litter, and sex ratio (both for data at birth and at weaning).

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EXPECTED FREQUENCIES OF CHROMOSOME ASSOCIATIONS IN TETRAPLOIDS WITH RANDOM CHIASMA FORMATION

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IN tetraploids the degree of multivalent formation at meiosis is often used as a criterion of homology between the chromosome sets and gives information on the genetic control of chromosome association. Where the chiasma frequency of each association can be recorded in a number of cells, the observed association frequencies could be profitably compared with frequencies expected, assuming they arise from the random formation of chiasmata between the four chromosomes. Even where the chiasma frequencies are not easily obtained, it would be an advantage to have some idea of the association frequencies expected on this basis. Some formulae have been derived and tables prepared for this purpose.

Association frequencies

The four types of association are: one bivalent and two univalents (II.2I); two bivalents (II.II); one trivalent and one univalent (III.I); and one quadrivalent (IV). Each chiasma formed at random among a set of four chromosomes can occur in one of six ways. The formation of one chiasma can give only one result, namely II.2I. Two chiasma can give three different associations having the frequencies,

II.2I	$\frac{1}{6}$
III.I	$\frac{4}{6}$
II.II	$\frac{1}{6}$

If another chiasma is formed these associations in turn give rise to the association frequencies,

II.2I	II.2I	$\frac{1}{6}$
	III.I	$\frac{4}{6}$
	II.II	$\frac{1}{6}$

III.I
- III.I $\frac{3}{6}$
- IV $\frac{3}{6}$

II.II
- II.II $\frac{2}{6}$
- IV $\frac{4}{6}$

More generally, the above associations always give rise to these association frequencies when another chiasma is formed, and association IV remains unchanged. This is shown by the chiasma matrix, Table 1, where p, a positive integer, is the

TABLE 1

Chiasma matrix

	w_p	x_p	y_p	z_p
w_{p+1}	1/6	...	...	..
x_{p+1}	1/6	1/3	...	..
y_{p+1}	2/3	...	1/2	..
z_{p+1}	...	2/3	1/2	1

number of chiasmata formed between any set of four chromosomes, and w_p, x_p, y_p, z_p are the frequencies of II.2I, II.II, III.I, and IV respectively, when p chiasmata are formed. When $p = 1$, then $w_p = 1$ and $x_p = y_p = z_p = 0$. The expected frequencies for any value of p may be derived as follows:

II.2I This association occurs only when each additional chiasma is formed on this same association. Therefore,

$$w_p = \frac{1}{6} w_{p-1} = \left(\frac{1}{6}\right)^{p-1} w_1$$
$$= \left(\frac{1}{6}\right)^{p-1} \text{ since } w_1 = 1.$$

II.II Neither this association nor II.2I can occur when another chiasma is formed on III.I or IV, and

$$x_p = \frac{1}{6} w_{p-1} + \frac{2}{6} x_{p-1}$$
$$= \left(\frac{1}{6}\right)^{p-1} w_1 + \frac{2}{6}\left(\frac{1}{6} w_{p-2} + \frac{2}{6} x_{p-2}\right)$$

$$= \left(\frac{1}{6}\right)^{p-1} w_1 + 2\left(\frac{1}{6}\right)^{p-1} w_1 + 2^2\left(\frac{1}{6}\right)^2 x_{p-2}$$
$$= \left(\frac{1}{6}\right)^{p-1} w_1 + 2\left(\frac{1}{6}\right)^{p-1} w_1 + 2^2\left(\frac{1}{6}\right)^{p-1} w_1 + 2^3\left(\frac{1}{6}\right)^3 x_{p-3}$$
$$= \left(\frac{1}{6}\right)^{p-1} w_1\left(1 + 2 + 2^2 + 2^3 \ldots . + 2^{p-2}\right) + 2^{p-1}\left(\frac{1}{6}\right)^{p-1} x_1.$$

But $w_1 = 1$ and $x_1 = 0$, so that

$$x_p = \left(\frac{1}{6}\right)^{p-1}\left(1 + 2 + 2^2 + 2^3 \ldots . + 2^{p-2}\right)$$
$$= \left(\frac{1}{6}\right)^{p-1}\left(2^{p-1} - 1\right).$$

III.I Neither this association nor II.2I can occur when another chiasma is formed on II.II or IV, and

$$y_p = \frac{4}{6} w_{p-1} + \frac{3}{6} y_{p-1}$$
$$= 4\left(\frac{1}{6}\right)^{p-1} w_1 + \frac{3}{6}\left(\frac{4}{6} w_{p-2} + \frac{3}{6} y_{p-2}\right)$$
$$= 4\left(\frac{1}{6}\right)^{p-1} w_1 + 4 \times 3\left(\frac{1}{6}\right)^{p-1} w_1 + 3^2\left(\frac{1}{6}\right)^2 y_{p-2}$$
$$= 4\left(\frac{1}{6}\right)^{p-1} w_1 + 4\times 3\left(\frac{1}{6}\right)^{p-1} w_1 + 4\times 3^2\left(\frac{1}{6}\right)^{p-1} w_1 + 3^3\left(\frac{1}{6}\right)^3 y_{p-3}$$
$$= 4\left(\frac{1}{6}\right)^{p-1} w_1\left(1 + 3 + 3^2 + 3^3 \ldots . + 3^{p-2}\right) + 3^{p-1}\left(\frac{1}{6}\right)^{p-1} y_1$$

But $w_1 = 1$ and $y_1 = 0$, so that

$$y_p = 4\left(\frac{1}{6}\right)^{p-1}\left(1 + 3 + 3^2 + 3^3 \ldots . + 3^{p-2}\right)$$
$$= 2\left(\frac{1}{6}\right)^{p-1}\left(3^{p-1} - 1\right).$$

IV The frequency of quadrivalents is found by subtracting the sum of the other frequencies from one, and algebraically this is

$$z_p = \left(\frac{1}{6}\right)^{p-1}\left(2^{p-1} - 2\right)\left(3^{p-1} - 1\right).$$

The frequencies of the four types of association are shown in Table 2 for values of p up to 12. With three chiasmata less than half the associations are quadrivalents and at least six chiasmata are required for more than 95 percent to be quadrivalents.

A deficiency of quadrivalents implies localization of chiasmata on certain chromosomes. In particular, excess of II.2I and II.II indicates some measure of allopolyploidy which can be tested by combining these two classes to give an expectation of $\left(\frac{1}{3}\right)^{p-1}$, leaving $1 - \left(\frac{1}{3}\right)^{p-1}$ for the combined expectation of the remaining two classes. These two combined class expectations are also the ex-

TABLE 2

Association frequencies percent

p	II.2I	II.II	III.I	IV
1	100	....	....	...
2	16.67	16.67	66.67	....
3	2.78	8.33	44.44	44.44
4	.46	3.24	24.07	72.22
5	.08	1.16	12.35	86.42
6	.01	.40	6.22	93.36
7	....	.14	3.12	96.74
8	....	.05	1.56	98.39
9	...	.02	.78	99.20
10	...	....	.39	99.60
11	....	....	.18	99.82
12	....	....	.10	99.90

pected frequencies of the two types of association occurring in triploids, II.I and III respectively, so that a simple comparison can be made between the triploid and tetraploid.

Poisson and normal distributions of p

It is not legitimate to compare the observed total frequencies for all values of p with expected frequencies calculated from the mean chiasma frequency, $\bar{p}$, of the data, but an over-all comparison can be made if the distribution of p is taken into account when calculating the expected frequencies. In practice, the distribution of p is supplied by the data but here expected total frequencies have been calculated, assuming a Poisson distribution where the variance is equal to the mean (Table 3), and assuming a normal distribution with the variance equal to one third of the mean (Table 4). The two tables are essentially the same. With a mean chiasma frequency of four, about 60 percent of the association are quadrivalents, and about 75 percent with a mean chiasma value of five. The maximum frequency of double bivalents, II.II, is never more than ten percent, and generally they are much less frequent than this. Evidently with random chiasma formation the allopolyploid type of association is comparatively rare.

TABLE 3

Total association frequencies percent (Poisson distribution)

$\bar{p}$	4I	II.2I	II.II	III.I	IV
1	36.8	40.0	3.6	15.4	4.1
2	13.6	32.1	6.3	28.5	19.4
3	5.0	19.4	6.3	30.6	38.7
4	1.8	10.5	4.9	25.9	56.8
5	0.6	5.3	3.4	19.6	71.1
6	0.3	2.6	2.2	13.8	81.1

TABLE 4

Total association frequencies percent (normal distribution)

$\overline{p}$	4I	II.2I	II.II	III.I	IV
1	19.0	65.2	3.2	12.6	...
2	3.0	32.5	9.7	41.8	12.9
3	1.0	11.2	8.0	39.5	40.3
4	...	3.0	4.7	27.6	64.6
5	...	0.7	2.3	16.8	80.1
6	...	0.3	1.2	9.8	88.7

TABLE 5

Bivalent frequencies percent (in association II.II)

	Number of chiasmata per bivalent								
p	1	2	3	4	5	6	7	8	9
2	100.0	...	...	...	...	...	...	...	...
3	50.0	50.0	...	...	...	...	...	...	...
4	28.6	42.8	28.6	...	...	...	...	...	...
5	16.7	33.3	33.3	16.7	...	...	...	...	...
6	9.7	24.2	32.3	24.2	9.7	...	...	...	...
7	5.6	16.7	27.8	27.8	16.7	5.6	...	...	...
8	3.1	11.0	22.0	27.6	22.0	11.0	3.1	...	...
9	1.8	7.1	16.5	24.7	24.7	16.5	7.1	1.8	...
10	1.0	4.4	11.7	20.5	24.7	20.5	11.7	4.4	1.0

Chiasma frequencies in the bivalents

Random chiasma formation among the bivalents of association II.II can be tested by comparing the frequencies of bivalents with 1, 2, 3, ... $p-1$ chiasmata with those listed in Table 5. If p chiasmata are distributed at random over the two bivalents and each chiasma has an equal chance of occurring between the two chromosomes of either of the two bivalents, then the probability of 0, 1, 2 .... p chiasmata occurring between the chromosomes of either of the two bivalents is given by the expansion of the binomial $\left(\frac{1}{2}+\frac{1}{2}\right)^p$.

$\left(\frac{1}{2}\right)^p$ bivalents have no chiasmata, and an equal proportion have p chiasmata which together form association II.2I. This has a frequency of $\left(\frac{1}{2}\right)^{p-1}$. The remainder of the terms of the binomial expansion when divided by $1-\left(\frac{1}{2}\right)^{p-1}$ give the frequencies of bivalents with 1, 2, 3 .... $p-1$ chiasmata in association II.II (Table 5).

SUMMARY

Formulae and tables of association frequencies in tetraploids are set out assuming that the associations arise from the random formation of chiasmata between the four chromosomes.

INDUCED SOMATIC SEGREGATION IN ASPERGILLUS NIDULANS

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HETEROZYGOUS diploid strains of *Aspergillus nidulans* spontaneously produce segregant clones belonging to three classes: diploid segregants which have undergone mitotic crossing over; haploid segregants with random redistribution of whole chromosomes (PONTECORVO, TARR GLOOR and FORBES 1954); and "nondisjunctional" segregants with all the markers on both branches of one chromosome in the homozygous condition, while the other chromosomes are still in the heterozygous condition (PONTECORVO and KAFER 1958). IKEDA, ISHITANI and NAKAMURA (1957) observed increases in mitotic segregation due to ultraviolet irradiation in some diploid strains of *Aspergillus oryzae*. MORPURGO and SERMONTI (1959) increased the frequency of mitotic segregation in some heterozygous diploid strains of *Penicillium chrysogenum* by the use of irradiation and chemical mutagenic agents.

It does not appear from the work published so far what types of chromosomal rearrangement are induced by mutagenic agents in diploid molds. This paper describes a preliminary investigation of the rearrangements produced by three mutagenic agents, formaldehyde, nitrogen mustard, and ultraviolet irradiation, on a heterozygous diploid of *Aspergillus nidulans*, marked on six out of the eight chromosomes so far identified (KAFER 1958).

MATERIAL AND METHODS

The cultures and microbiological techniques adopted as routine by PONTECORVO and his colleagues (1954) are used throughout, except where otherwise indicated.

Strain: The strain used was a diploid strain of *Aspergillus nidulans* kindly supplied by MR. E. FORBES of the University of Glasgow. The diploid is green and prototrophic, and its genotype is as follows:

$$\text{I}\ \frac{su_1 ad_{20}}{+} \bigcirc \frac{y\ ad_{20}\ +}{+\ +\ bi}; \qquad \text{II}\ \frac{Acr_1\ +}{+\ w}; \qquad \text{III}\ \frac{phen_2\ +}{+\ s_{12}};$$

$$\text{IV}\ \frac{pyro_4\ +}{+\ orn_1}; \qquad \text{V}\ \frac{lys_5\ +}{+\ nic_2}; \qquad \text{VII}\ \frac{nic_8}{+}.$$

PONTECORVO and KAFER's (1958) symbols for the mutant alleles and also their numbering of the chromosomes have been used. The symbol system and the

method of tabulating data adopted by PONTECORVO and KAFER (1958) have been followed as far as possible in the present paper in order to simplify the comparison between the results now obtained concerning induced mitotic recombination and those of the earlier writers concerning spontaneous mitotic recombination. The symbol for mutant su_1ad_{20} (ad_{20} suppressor) will be abbreviated to *su*; marker Acr_1 was not considered. All the other markers are nutritional requirements, except those referred to as *y* and *w*, yellow and white conidium color respectively (*w* is epistatic to *y*). Marker nic_2 was not distinguished from nic_8 (nicotinamide requirements).

Medium: The minimal medium (Czapek-Dox) and the complete medium (corn-steep-sucrose) were adopted as standard in this laboratory (SERMONTI 1957). Three gm/litre of yeast extract were added to the complete medium.

Mutagenic agent treatment: Three mutagenic agents were used: formaldehyde (HCHO), methyl-bis(β-chloroethyl)amine (HN-2), and ultraviolet irradiation (U.V.). The most extensively used was HCHO, which in the majority of the experiments was added to the complete agar. Its action was continuous throughout colonial growth. HN-2 and U.V. were used on freshly collected conidia. The use of these two agents has been described in earlier papers (SERMONTI 1956).

Selection and classification of somatic segregants: Somatic segregants were selected for yellow or white color as sectors or macroscopic spots visible on the green colonies of the heterozygous diploid and in some cases as whole yellow or white colonies. Not more than one yellow and one white segregant were collected from each colony. The segregant clones were purified and then tested for nutritional requirements. Every colony tested which failed to develop on a dish lacking a particular nutrient was considered to have a requirement for that nutrient.

Classification criteria

Determination of ploidy of segregants

The possibility of stable aneuploid conditions was disregarded in accordance with the views of PONTECORVO and KAFER (1958); isolated and purified segregants have, therefore, been classified as either diploid or haploid. This assumption cannot be proved or disproved by the analytical method used in this paper. In particular, in all the segregants which will here be regarded as diploids, it is not determined whether the segregant region is in the homozygous or hemizygous state.

Haploid segregants: Segregants with the markers of one or other of the members of each of the chromosome pairs in both parents were considered haploid. As appears from Table 1, two thirds of the identifiable haploid segregants express phenotypically the markers of only four chromosomes, and one third those of five chromosomes. The segregation of chromosome VII was not taken into account since its marker, nic_8, mimics nic_2 on chromosome V. In half the cases the segregation of chromosome I is masked because *w* is epistatic to *y*, and in half the cases the segregation of chromosome II was not detected because marker Acr_1 was ignored. Where there is no evidence that a chromosome pair has failed to segregate, all the chromosomes are assumed to have segregated. Such segregants

TABLE 1

Phenotype, deduced genotype, and frequency of identified haploid segregants induced by formalin

Phenotypes*	Frequency†	Genotypes — Linkage groups I				II		III		IV		V	
		su	*y*	ad_{20}	+	Acr_1	+	$phen_2$	+	$pyro_4$	+	lys_5	+
		+	+	+	*bi*	+	*w*	+	s_{12}	+	orn_4	+	nic_2
w phen pyro lys (nic)	16	*su*	*y*	ad_{20}	+	+	*w*	$phen_2$	+	$pyro_4$	+	lys_5	+
w phen pyro nic	20	*su*	*y*	ad_{20}	+	+	*w*	$phen_2$	+	$pyro_4$	+	+	nic_2
w phen orn lys (nic)	1	*su*	*y*	ad_{20}	+	+	*w*	$phen_2$	+	+	orn_4	lys_5	+
w phen orn nic	2	*su*	*y*	ad_{20}	+	+	*w*	$phen_2$	+	+	orn_4	+	nic_2
w s pyro lys (nic)	10	*su*	*y*	ad_{20}	+	+	*w*	+	s_{12}	$pyro_4$	+	lys_5	+
w s pyro nic	22	*su*	*y*	ad_{20}	+	+	*w*	+	s_{12}	$pyro_4$	+	+	nic_2
w s orn lys (nic)	3	*su*	*y*	ad_{20}	+	+	*w*	+	s_{12}	+	orn_4	lys_5	+
w s orn nic	5	*su*	*y*	ad_{20}	+	+	*w*	+	s_{12}	+	orn_4	+	nic_2
bi w phen pyro lys (nic)	11	+	+	+	*bi*	+	*w*	$phen_2$	+	$pyro_4$	+	lys_5	+
bi w phen pyro nic	4	+	+	+	*bi*	+	*w*	$phen_2$	+	$pyro_4$	+	+	nic_2
bi w phen orn lys (nic)	0	+	+	+	*bi*	+	*w*	$phen_2$	+	+	orn_4	lys_5	+
bi w phen orn nic	5	+	+	+	*bi*	+	*w*	$phen_2$	+	+	orn_4	+	nic_2
bi w s pyro lys (nic)	13	+	+	+	*bi*	+	*w*	+	s_{12}	$pyro_4$	+	lys_5	+
bi w s pyro nic	20	+	+	+	*bi*	+	*w*	+	s_{12}	$pyro_4$	+	+	nic_2
bi w s orn lys (nic)	1	+	+	+	*bi*	+	*w*	+	s_{12}	+	orn_4	lys_5	+
bi w s orn nic	2	+	+	+	*bi*	+	*w*	+	s_{12}	+	orn_4	+	nic_2
y phen pyro lys (nic)	11	*su*	*y*	ad_{20}	+	Acr_1	+	$phen_2$	+	$pyro_4$	+	lys_5	+
y phen pyro nic	12	*su*	*y*	ad_{20}	+	Acr_1	+	$phen_2$	+	$pyro_4$	+	+	nic_2
y phen orn lys (nic)	4	*su*	*y*	ad_{20}	+	Acr_1	+	$phen_2$	+	+	orn_4	lys_5	+
y phen orn nic	2	*su*	*y*	ad_{20}	+	Acr_1	+	$phen_2$	+	+	orn_4	+	nic_2
y s pyro lys (nic)	8	*su*	*y*	ad_{20}	+	Acr_1	+	+	s_{12}	$pyro_4$	+	lys_5	+
y s pyro nic	15	*su*	*y*	ad_{20}	+	Acr_1	+	+	s_{12}	$pyro_4$	+	+	nic_2
y s orn lys (nic)	2	*su*	*y*	ad_{20}	+	Acr_1	+	+	s_{12}	+	orn_4	lys_5	+
y s orn nic	2	*su*	*y*	ad_{20}	+	Acr_1	+	+	s_{12}	+	orn_4	+	nic_2

* The symbol (nic) implies that the corresponding frequencies are the sum of classes with and without a nicotinic acid requirement.
† These are the pooled frequencies from all the experiments with formalin.

will be called *genomic*, in line with the terms used for the classes considered below.

Diploid segregants: Segregants showing markers located on only one chromosome were regarded as diploid. One of the first pair of chromosomes has markers on both its arms, *su* on the left and *y* and ad_{20} on the right. The latter two will be regarded as being located at a single locus, being only 0.2 units apart (PONTECORVO and KAFER 1958). Simultaneous segregation of all the markers on chromosome I produces yellow prototrophic segregants, whereas segregation of the markers on the right arm only gives yellow adenineless segregants. The first type will be called *chromosomal segregants* and the second type *subchromosomal segregants* throughout. According to PONTECORVO and KAFER (1958), the first type derives from nondisjunction, the second from mitotic crossing over. We prefer to use a formal designation of the two types of segregants, without reference to the processes from which the segregants arise, since the somatic segregants we are dealing with are of induced origin, and processes different from those described by PONTECORVO and KAFER (1958) may also produce the same segregant phenotypes. In particular it is not possible to phenotypically distinguish the results of mitotic crossing over from those of terminal chromosome deletions, and the latter are to be expected frequently after mutagenic treatments. Segregants showing markers from two different pairs of chromosomes are also regarded as diploid, but those showing markers from more than one pair are counted as "aberrant" (or classified as haploid segregants) if they fulfill the requirements shown above for such classification.

Other segregants: These include segregants showing markers from three chromosome pairs—those showing markers from both members of the same pair, and others whose origin can only be explained on the assumption that different segregation events have taken place simultaneously. They will be discussed in the next section.

RESULTS AND DISCUSSION

Effect of formaldehyde treatment

Treatment of conidia: In some experiments, conidia of the heterozygous diploid were suspended in distilled water containing HCHO at a concentration of 0.05 percent (v/v) for periods from one to 13 minutes, giving survival rates of from 30 percent to 0.04 percent of the conidia. In none of the conditions tried did any increase of somatic segregation appear in the surviving colonies.

Treatment of growing colonies on agar: A more extensive study was made of the effect of HCHO on colonies grown on complete agar containing the mutagenic agent in concentrations of 0.05 percent, 0.04 percent, or 0.03 percent (Figure 1). At the highest concentration (experiments 1336, 1305) only 1–2 percent of the conidia seeded produced colonies; at the lowest concentration (experiment 1331) more than half the conidia produced colonies. In the absence of HCHO the macroscopically visible segregants show up as narrow strips comprising a few tens of white or yellow heads, detectable in about a tenth of the colonies. In the presence

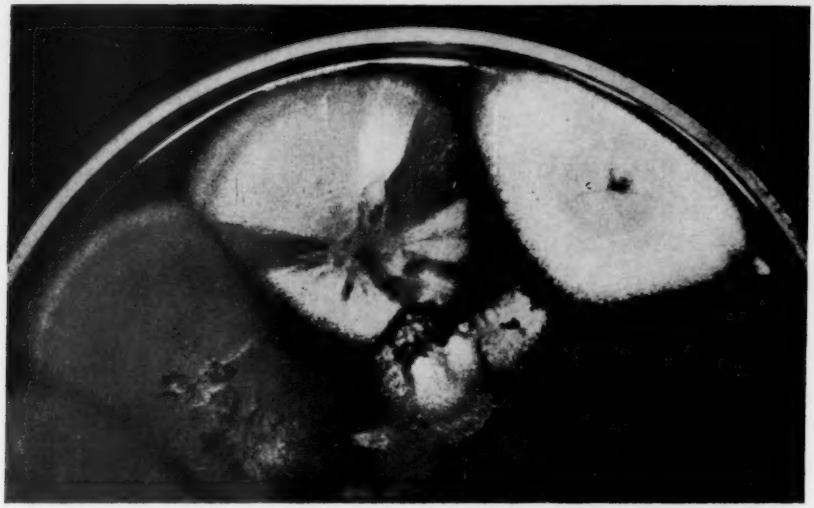

FIGURE 1.—Color segregation in colonies growing on agar containing 0.05 percent formalin.

of HCHO, the pattern changes as the concentration is increased. At low concentrations, the majority of the colonies grew in the presence of HCHO not appreciably slower than normal; only a small percentage of ("anomalous") colonies grew poorly, remaining small in size and producing practically no conidia; the appearance of the sectoring colonies was essentially the same as in the absence of HCHO, but they were more numerous. At the highest concentrations, "anomalous" colonies appeared in large numbers; it was not possible to classify these as sectoring or nonsectoring. On transferring them to fresh medium without HCHO, practically all these colonies produced colored sectors. These "anomalous" colonies constituted more than 50 percent of the whole at the highest concentrations (experiment 1336). Alongside these there were normally growing colonies with wide colored sectors or broad stripes, and completely white or yellow colonies, generally characterized by a dense conidiumless central zone of a deep brown color on the back.

The sectors, and the white or yellow segregant areas, were suitably purified and classified according to the criteria already described for phenotype and ploidy. Eight experiments have been arranged into four groups (Table 2) according to the survival rates of the conidia. These will be taken hereafter as measure of the intensity of the effect of the mutagenic agent. Four increasing "doses" of HCHO—from one to four—will be considered, corresponding to the four groups of decreasing survival rates. The "doses" are roughly proportional to the actual concentrations of HCHO in agar (Table 2).

Haploid (or genomic) segregants: Their relative frequency increases in proportion with the increasing of the mortality of the conidia plated. (Table 2 and

TABLE 2

Experiments in formalin-induced somatic segregation

Experiments* (code no.)	Formalin conc. in agar (percent, v/v)	HCHO "dose"	Survival (percent)	Colonies observed (no.)	Colonies exhibiting segregation (percent)†	Classification of segregants								
							Diploid							
						Color	Segregation in one chromosome (adenine requir.)	(no.)	Segregation in two chromosomes (no.)	Totals (no.)		Haploid (no.)	Aberrant§ (no.)	Total (no.)
1336–1305	0.05	4	1.1–1.8	892	93.1‡	yellow	+	15	10	25	38	39	15	92
							ad	11	2	13				
						white		23	14	37		102	28	167
						totals		49	26	75		141	43	259
1356a–1351a	0.04	3	15–18	75	91.5	yellow	+	5	2	7	12	3	2	17
							ad	5	0	5				
						white		8	2	10		7	4	21
						totals		18	4	22		10	6	38
1351b–1330	0.03–0.04	2	38–40	265	50.0	yellow	+	7	6	13	39	10	2	51
							ad	25	1	26				
						white		20	3	23		17	4	44
						totals		52	10	62		27	6	95
1356b–1331	0.03	1	49–59	340	34.9	yellow	+	2	1	3	39	4	0	43
							ad	32	4	36				
						white		16	1	17		9	2	28
						totals		50	6	56		13	2	71

* The experiments have been grouped according to the survival rate.
† Percent of scorable colonies. "Anomalous" colonies not considered.
‡ Data from experiment 1336.
§ Unclassifiable as haploid or diploid (see text).

Figure 2, left). At the highest dose of HCHO (experiments 1336, 1305) the genomic segregants represent more than 50 percent of all tested segregants, as against less than 20 percent at the lowest dose (experiments 1356b, 1331).

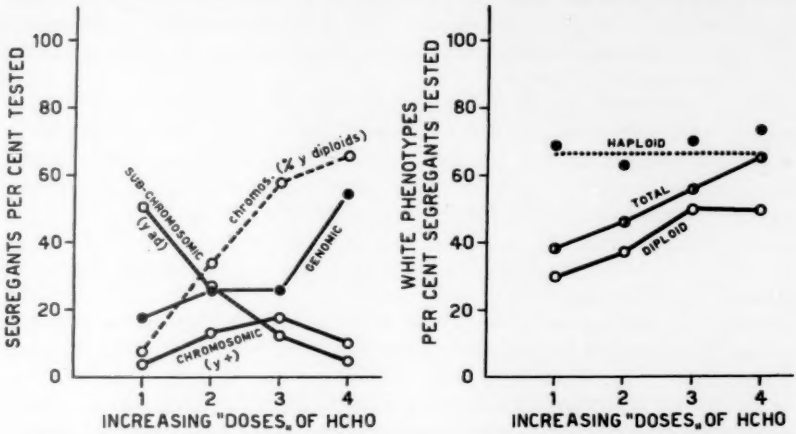

FIGURE 2.—Graphical representation of some frequency values deduced from Table 2. (Left) Frequencies of different classes of segregant percent color segregants tested. The circles joined by the broken line give the frequencies of chromosome segregants ($y + ^{ad}$) percent yellow diploid segregants tested. (Right) Frequencies of white segregants percent haploid, diploid or total segregants tested. The dotted line is the expected value for the haploid segregants (2/3).

Markers located on different chromosomes recombine at random (Table 3). For each pair of chromosomes, the frequency of the recombinant classes is almost equal to the frequency of the parental classes, i.e., to half the total frequency of

TABLE 3

Mitotic recombination (percent) in formalin-induced haploid segregants between markers of different linkage groups

Linkage groups	I		II		III	IV	VII
	Actual	Corrected*	Actual	Corrected†			Corrected‡
V	55.0	52.8	45.0	46.9	42.9	54.4	47.1
IV	36.2	47.4	66.0	55.2	53.4	...	...
III	45.5	43.3	45.0	45.8	...	...	...
II	...	58.0§	...	...	...	...	...

* The member of chromosome pair I marked with *su y* ad_{29} occurs in two thirds of the selectable haploid segregants, and the member with *bi* in one third (Table 1). The figures have been corrected by doubling the frequency of the biotine requiring segregants.

† The member of chromosome pair II marked with *w* occurs in two thirds of the selectable haploid segregants and the member with Acr_1 in one third. The figures have been corrected by doubling the frequency of the non *w* (yellow) haploids.

‡ Only the recombinant class is detectable ($lys+^{ade}$). The recombinant frequency has been obtained by doubling the frequency of this class and dividing by the total haploids.

§ Two parental classes are selectable and only one recombinant class. The frequency of the latter has therefore been doubled to calculate the recombination frequency.

the selected haploids. This of course does not apply to the recombinant classes between the first and second chromosome, which carry the markers (y and w) for which the selection was made. Only three of the four combinations (YW, yW, Yw, yw) can be selected, one recombinant (yw) and the two parental (yW and Yw) (Table 1). Among the haploid segregants the number of yellow segregants (yW) is always about half of the number of white segregants ($yw + Yw$) (Table 2 and Figure 2, right).

Diploid segregants: Diploid segregants form a smaller proportion of all the segregants tested as the dose of HCHO increases (Table 2): from about 80 percent at the lower concentrations (experiments 1356b, 1331) to 34 percent at the highest concentrations (experiments 1336, 1305). As already mentioned in the section headed "Classification criteria," there are two distinct classes of diploid segregants showing markers in coupling with y in chromosome I—chromosomal ($su\ y\ ad_{20}$) and subchromosomal ($y\ ad_{20}$). The frequency of chromosomal segregants among yellow diploids increases very greatly as the HCHO concentration in the agar increases, until at the highest concentrations they are in the majority, whereas they are less than ten percent at the lowest concentrations (Figure 2, left).

A proportion of the diploid segregants express markers segregating on a second chromosome besides the one with the selected marker (Table 2). Since none of the chromosomes without selective markers is marked on both branches, it is impossible to distinguish between chromosomal and subchromosomal segregation of the unselected markers. The same is true of the chromosome carrying the w marker.

The coincidence of segregation on two chromosomes varies from about ten percent of diploid segregants in the cultures treated with dose one of HCHO to 35 percent in those treated with dose 4 (Table 2).

Aberrant phenotypes: Some segregants deviate from the phenotypes classifiable as haploid by having one marker from each member of one pair of chromosomes, alongside one marker from either member of the other chromosome pairs. These segregants can be classified with certainty as haploids with one crossover chromosome carrying both the markers of the parent chromosomes.

Other segregants deviate from the haploid phenotypes (Table 1) by the absence of one or two markers. Assuming not more than one crossing over simultaneous with haploidization, and not more than three chromosomes segregating simultaneously in segregant diploids, these can be interpreted as falling into three classes (Table 4):

1. Diploids segregating for three chromosomes;

2. Haploids with one crossover chromosome (phenotype deviating from the haploid phenotypes with evidence of segregation on five or six chromosomes (see Table 1), the markers of one or of two chromosomes respectively being absent);

3. Optional, i.e., possibly belonging to either of the above classes (phenotype deviating from the haploid phenotypes with evidence of segregation on four chromosomes (see Table 1), the markers of one chromosome being absent).

TABLE 4

Aberrant phenotypes obtained after formalin treatment (experiment 1305)

(1) (Diploid?) segregants for three chromosomes		(2) Haploid segregants with one crossover chromosome		(3) Optional segregants	
Phenotypes	(no.)	Phenotypes	(no.)	Phenotypes	(no.)
bi w phen	1	w pyro lys nic*	2	w s nic	1
		bi w s nic	1	w phen nic	1
		bi w phen pyro	1	w pyro nic	2
		bi w phen lys	1	w pyr lys	1
		bi w phen nic	1	y s nic	2
		bi w pyro lys*	1	y s lys	1
		bi w *phen s* pyro nic †	1	y phen lys	1
		bi w *phen s* pyro lys nic†	2	y phen nic	1
		bi w *phen s* lys nic†	1		

* Recessive markers from both members of chromosome III (*phen, s*) absent.
† Recessive markers from both members of chromosome III (*phen, s*) present.

In the latter haploids (classes 2 and 3) the crossover chromosome is assumed to carry the wild alleles of the markers of both parental chromosomes. The aberrant haploids with one marker in excess may be classified with those of class 2 (Table 4).

The most frequent crossover chromosome among aberrant phenotypes is one carrying the markers $phen_2$ and s_{12}. There are eight of these (4.7 percent of haploids) among all the HCHO-induced segregants, and both markers are missing in 13 of the haploid or optional segregants (classes 2 and 3). Probably some of this last type are haploid, carrying the crossover chromosome complementary to $phen_2$-s_{12} and some diploids segregant on three chromosomes not including chromosome III.

Effect of nitrogen mustard and ultraviolet irradiation treatment

As in the case of the HCHO treatments, the experiments have been set in groups, according to the survival rates. Only two groups for each mutagenic agent were set due to the small crop of data so far collected. They were considered as treated with dose 1 (higher survival) or dose 2 (lower survival) of the corresponding agent (Table 5).

HN-2: No evaluation was made of the frequency of anomalous colonies, which in this case do not form a clearly defined class. The frequency of sector forming colonies is related to the total of surviving colonies. In the experiment 1373a (Table 5) it was over 53.6 percent. This figure is not comparable with the frequency obtained after formalin treatment, in view of the different conditions of treatment. The percentage of haploid segregants is higher at the highest dose (2) of HN-2. At this dose the number of chromosomal segregants becomes greater than that of subchromosomal segregants (see "Classification criteria" and the section on the action of formalin); the coincidence of segregation of two chromosomes was 32 percent. The segregation pattern at the highest HN-2 dose was very similar to that at the highest dose of formalin, but there is no pattern correspond-

TABLE 5

Experiments in nitrogen mustard and ultraviolet irradiation induced and spontaneous somatic segregation

Experiments* (code no.)	Survival (percent)	Colonies observed (no.)	Colonies exhibiting segregation (percent)†	Classification of segregants								
					Diploid				Haploid (no.)	Aberrant‡ (no.)	Total (no.)
				Color	Segregation in one chromosome (adenine requir.)	(no.)	Segregation in two chromosomes (no.)	Totals (no.)			
				(1) Nitrogen mustard (HN-2)							
1373a, 1370, 1373b	0.6, 1.8, 4.0	163	46.0	yellow	+	8	4	12	18	13	15	46
					ad	5	1	6				
				white		8	5	13		38	4	55
				totals		21	10	31		51	19	101
1389	23.0	218	28.4	yellow	+	5	1	6	16	7	2	25
					ad	8	2	10				
				white		2	2	4		19	1	24
				totals		15	5	20		26	3	49
				(2) Ultraviolet irradiation (U.V.)							
1379a, b,	2.3, 2.5	356	18.5	yellow	+	2	0	2	5	7	2	14
					ad	3	0	3				
				white		5	1	6		13	4	23
				totals		10	1	11		20	6	37
1379a, b,	7.5, 14.0	196	21.0	yellow	+	3	1	4	15	8	1	24
					ad	10	1	11				
				white		4	0	4		10	1	15
				totals		17	2	19		18	2	39
				(3) Spontaneous							
1364, 1374 1389, 1402	.....	690	12.6	yellow	+	3	0	3	35	8	1	44
					ad	32	0	32				
				white		23	4	27		17	0	44
				totals		58	4	62		25	1	88

* The experiments have been grouped according to the survival rate.
† Percent of colonies observed.
‡ Unclassifiable as haploid or diploid.

ing to that at the lower doses of formalin. At dose 1 (survival 23.0 percent) only four percent of the surviving colonies had subchromosomal segregants, the same percentage as in spontaneous segregation. Thus, there is as yet no experimental evidence to show that HN-2 increases the rate of subchromosomal segregation. Out of a total of 22 aberrant phenotypes, only one had the crossover chromosome *phen-s* while seven lacked both these markers.

U.V. rays: The data obtained are hardly conclusive. The pattern seems not to differ from that obtained with nitrogen mustard. At equal lethality the stimulation of segregation would seem to be much less effective. This is shown by the lower rate of colonies showing segregation and by the lower coincidence.

Evaluation of segregation frequencies

The mutagenic action of formalin was exerted on nuclei in division (not on resting nuclei), and it is difficult to establish a parameter by which to calculate the segregation rate. As an index of the intensity of segregation the frequency of colonies exhibiting yellow or white sectors has been adopted (the "anomalous" colonies have been disregarded—see Table 2). Since this frequency is equal to 12.6 in the untreated colonies (Table 5), the observable increase in segregation cannot exceed eightfold (100:12.6). This is actually 7.4 fold with the highest dose of formalin. Apart from the limitation of the method, this value is certainly conservative, since in the presence of formalin the number of sectors per sectoring colony is much higher than in the absence of the drug. This number, however, cannot be safely estimated. Evaluation of the frequency of segregation induced by HN-2 (or U.V.) is simpler than with HCHO induced segregation. Since the material treated consists of single resting uninucleate conidia, the effect can easily be evaluated as percent of affected colonies among those surviving the treatment. In experiment 1377a more than 50 percent exhibited symptoms of the action of HN-2 on their chromosomal integrity or nuclear kinetics. HN-2 has to a large extent a delayed effect, resulting in the formation of late sectors. U.V. action is less pronounced than that of HN-2. The observed effects are parallel to those reported by MORPURGO and SERMONTI (1959) in induced somatic segregation in *Penicillium chrysogenum* (Table 6). In that species the frequencies of the segregating colonies after treatment with the same three mutagenic agents dealt with in this paper are comparable with the frequencies obtained in *Aspergillus nidulans*, but the increase compared with the controls was much higher in some cases, macroscopically observable segregation being practically absent in the untreated colonies. The use as "control" of the rate of mitotic segregation in untreated colonies has only limited validity. What is called "spontaneous" segregation would be better described as segregation due to uncontrolled causes. It has been shown that the rate of spontaneous segregation may depend on genetic (MORPURGO and SERMONTI 1959; SERMONTI 1957) and environmental (SERMONTI and MORPURGO 1959b) factors. Rather it should be regarded as a background whose intensity determines the amount of information obtainable about induced segregation.

TABLE 6

Frequency of spontaneous and induced yellow segregants in Aspergillus nidulans *and* Penicillium chrysogenum

	Aspergillus nidulans				*Penicillium chrysogenum**				
Mutagenic agent	Surviving conidia (percent)	Colonies observed (no.)	Colonies exhibiting yellow segregants (no.)	(percent)	Strain	Surviving conidia (percent)	Colonies observed (no.)	Colonies exhibiting yellow segregants (no.)	(percent)
.....	(100)	690	87	12.6	XXXIV	(100)	1,103	44	4.0
					XXXIV S	(100)	623	0	0.0
Formalin (HCHO)	15–18	75	43	57.8	XXXIV	2.5	146	47	32.8
Nitrogen mustard (HN-2)	0.6	84	45	53.6	XXXIV	0.5	208	34	16.2
					XXXIV S	0.2	145	35	24.1
Ultraviolet rays (U.V.)	2.0–2.5	356	66	18.5	XXXIV	0.8	220	16	7.3

* After SERMONTI and MORPURGO (1959b).

One criterion for evaluating the increase in rate of diploid segregants after mutagenic agent action arises from consideration of the rate of coincident segregation of markers located on different chromosomes. In other words, the rate of segregation in the other chromosomes can be determined in segregants selected for segregation on one chromosome on the assumption of random simultaneous occurrence of the events considered. On this basis the increase in diploid segregants is about sixfold after HCHO or HN-2 treatment and barely detectable after U.V. treatment.

Figure 3 shows the frequencies as percent of colonies observed of the various classes of segregant sectors induced by increasing "doses" of HCHO. At zero dose the values shown are deduced from the figures for spontaneous segregation. Each frequency value in Figure 3 is calculated by multiplying the relative frequency (percent tested) of the various classes of segregant (Figure 2, left) by the frequency (percent observed) of colonies exhibiting color segregation (see Table 2 and Table 5, untreated).

The various types of segregant will be considered separately in relation to the action of HCHO.

Genomic (haploid) segregants: The frequency of genomic segregants is only slightly increased at HCHO doses sufficient to inactivate about half the conidia (dose 1). At higher doses it increases rapidly. As already noted, the number of white haploid segregants at all doses of HCHO is about double the number of yellow segregants (see Figure 2, right).

Chromosomal (nondisjunctional) segregants: The frequency of these is taken as equal to the frequency of colonies having yellow diploid segregants without adenine requirement (*su y* ad_{20}). The first part of the curve expressing this frequency follows the curve for the frequency of genomic segregation. This agrees with PONTECORVO and KAFER's (1958) proposal of a similar mechanism of origin for both types of segregant in spontaneous segregation. At higher HCHO doses there is a drop in the number of nondisjunctional sectors percent of surviving colonies, which may be related to the corresponding sharp increase in haploid

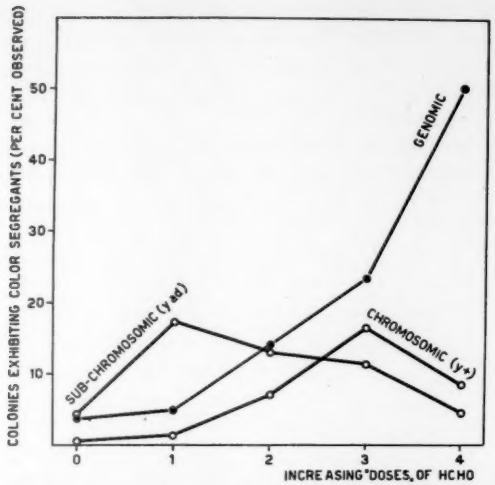

FIGURE 3.—Frequencies of colonies exhibiting color segregant sectors belonging to different classes. The values were obtained by multiplying the values given in Figure 2 (left) by the percent of colonies exhibiting segregation (Table 2, sixth column). The values for "dose" 0 (no HCHO in the agar) were calculated from Table 5 (spontaneous).

segregants; the process of irregular distribution of the chromosomes may tend, at higher doses of HCHO, to lead to haploidization rather than nondisjunction.

The relative increase in chromosomal segregation shows up in the relation between the frequencies of yellow and white diploid segregants. This should remain at about 2:1 so long as the only segregation process involved is crossing over (or other subchromosomal segregation process), since the *y* marker is about twice as far from the centromere as the *w* marker (meiotic distances; KAFER 1958). This ratio is actually found (2.29:1) at lower concentrations of HCHO (see Figure 2, right). As the concentration increases, the ratio comes closer to unity as is to be expected since the segregation frequency of the two markers in segregations involving whole chromosomes (nondisjunctional) is independent of the distances of the markers from the centromeres of their respective chromosomes. Since both genomic and chromosomal segregation produce more white segregants than subchromosomal processes, the over-all increase in white segregants as compared to yellow (see Figure 2, right) can be used as an index for the displacement of the segregation processes towards those due to irregular distribution of whole chromosomes, i.e., to aberration in centromere behavior. In other words, the extent of the excess of white over yellow segregants is diagnostic of disturbances in chromosome kinetics.

Subchromosomal segregants: The frequency of this class is taken as equal to the frequency of colonies showing yellow diploid segregants with an adenine requirement ($y\ ad_{20}$). This is highest at the lowest HCHO concentrations (dose 1), at which the increase in the frequency of other types of segregant is negligible.

As the HCHO dose increases (doses 2, 3, 4), the frequency of subchromosomal segregants decreases. This decrease is probably due to the reduced probability of collecting subchromosomal segregants as a consequence of the increase in other types of segregant and the precaution observed against collecting more than one segregant of the same color from any colony. This behavior suggests, however, that the processes leading to subchromosomal segregation are already stimulated by quite light treatment, while more drastic treatment produces aberrations affecting whole chromosomes. In comparing different classes of segregant sector it must be borne in mind that the ratio between detectable and undetectable segregants is very different for the various classes. Three quarters of the possible haploids are detectable. Only one chromosomic segregant of the 16 possible ones is characterizable. Detectable subchromosomic segregation would occur in only 20 meiotic units (from y to centromere) of a total of more than 1,000 (KAFER 1958). The different patterns of the three classes are therefore comparable, while the comparison between slopes is of no value.

It remains an open question whether or not subchromosomal segregation is due to somatic crossing over. There is at present no means of distinguishing between segregations due to crossing over, terminal deletions, and other breaches of chromosomal integrity. A point of some interest is the presence among the aberrant phenotypes of haploid segregants with the crossover chromosome showing two recessive markers which were originally in repulsion (Table 4). The *phen-s* segregants have already been considered. There were eight of these against 191 haploid HCHO-induced segregants without crossover, i.e., four percent. This figure gives an indication of the coincidence of somatic crossing over in the region concerned with haploidization. In these cases intrachromosomal recombination has certainly taken place. No crossover chromosomes were observed in the spontaneous haploid segregants. A comparison of somatic crossover frequencies in haploids and diploids, assuming subchromosomal segregation to be due to mitotic crossing over, cannot be made at present without too many other arbitrary assumptions.

Coincidence of different segregation events: The coincidence of different segregation events has already been considered in connection with segregation on two chromosomes and with haploidization accompanied by crossing over. One point which still has to be discussed is the possibility of segregation on more than two chromosomes. As already remarked, segregants for three chromosomes are not phenotypically distinguishable in many cases from haploid segregants with one crossover chromosome (see Table 4). The number of such segregants which have been detected with certainty is very small, but it remains possible that they may be quite frequent, since the majority of the aberrant segregants described as "optional" could belong to this class. This coincidence can only be regarded for the moment as a possible occurrence which will have to be borne in mind in future work.

Comparison of action of different agents: The segregation pattern produced by HN-2 (apart from the frequency of the induced segregation which is not comparable) corresponds to the "strong" effect of HCHO. There is the same lack of

evidence of any increase in subchromosomal segregation and the same almost complete absence of demonstrable crossover chromosomes in the haploid segregants (one *phen-s* chromosome out of 75 haploids).

The effect of U.V. is of the same type as that of HN-2, but the U.V. treatment is less effective at equal lethality levels. This is evident from both the small number of colonies with segregation and the small number of segregants with segregation on two chromosomes.

CONCLUSION

Emphasis should be laid upon the considerable amount of information obtainable from heterozygous diploid molds on the action of mutagenic agents at the chromosome level, and the convenience of this type of material for large-scale research. Microorganisms, including molds, have in the past been regarded as ideal material for the study of point mutations, but ill-adapted for the observation of chromosomal aberrations induced by mutagenic agents. The occurrence of heterozygous diploid molds and of induced somatic segregation make possible the use of microorganisms in the study of chromosomal mutations as well, and permit parallel research on the action of mutagenic agents at the gene and chromosome levels.

SUMMARY

The authors have studied the action of three mutagenic agents—formalin (HCHO), nitrogen mustard (HN-2), and ultraviolet rays (U.V.)—on somatic segregation processes in a heterozygous diploid of *Aspergillus nidulans*.

The following points are emphasized:

1. HCHO and HN-2 produced a sharp increase in the frequency of somatic segregation; this increase is less marked in the case of U.V.

2. HCHO treatment sufficient to give a survival rate of 50 percent produced a particularly notable increase in the frequency of subchromosomal segregants, possibly due to mitotic crossing over or terminal deletions. With increasing doses of HCHO increasing frequencies were obtained of chromosomal (nondisjunctional) and genomic (haploid) segregants, but no further increasing in the rate of subchromosomal segregants was observed.

3. The segregation patterns induced by HN-2 and U.V. treatment resemble that obtained at higher concentrations of HCHO; there is no indication of an increase in subchromosomal segregants.

This work illustrates the possibility of analysing genetically induced chromosomal rearrangements in a group of microorganisms.

ACKNOWLEDGMENTS

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MITOSIS IN VEGETATIVE NUCLEI OF NEUROSPORA CRASSA[1]

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FOR a number of years *Neurospora crassa* has been one of the most important materials for genetic research. During recent years it has been especially valuable in investigations on gene action at the biochemical level. Unfortunately, however, there exists a paucity of cytological knowledge of this ascomycete, which hinders correlations between genetic and cytological data. The most detailed accounts of chromosome morphology and the meiotic division cycle were presented by McCLINTOCK (1945, 1947) and by SINGLETON (1953). These authors, however, observed events occurring in developing asci with no descriptions concerning division phenomena of vegetative nuclei.

Recently TSUDA (1958) and SHATKIN (1959) studied the fine structure of cellular constituents of vegetative hyphae of *N. crassa*, including the nuclei. Their electronmicrographs revealed the nature of the nuclear membrane, but there was no evidence presented for nuclear division. Cinematographic observations of living cells have shown gross cellular behavior but, unfortunately, have not captured the sequences of mitosis because the motion of protoplasm obscured these events (HSU and FUERST 1956; FUERST and HSU 1957).

The first detailed account of the manner of division of somatic nuclei of *N. crassa* was presented by BAKERSPIGEL (1959), who in a series of articles on nuclear division of fungi maintains that "the nuclei do not appear to divide in the manner of classical mitosis. During division individually recognizable chromosomes were not seen to align themselves on a spindle or form a metaphase plate."

The present report describes preliminary observations on nuclear division occurring in the vegetative phase of a normal, wild type strain of *N. crassa*. It also gives an account of the number and morphology of metaphase chromosomes of this species.

MATERIALS AND METHODS

The strain of *Neurospora crassa* employed in this study was Em5256A (EMERSON and CUSHING 1946), a wild type strain which has been carried in our laboratories for a number of years. Crosses involving this strain were used as a standard by McCLINTOCK and SINGLETON in studying the chromosomal sequence occurring in the asci (SINGLETON 1953).

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Mature conidia were harvested from cultures which had been grown on complete agar media in test tubes. A heavy conidial suspension was made in minimal liquid medium (VOGEL 1956). This suspension was filtered through cheese cloth to remove mycelial fragments, and the conidia were allowed to germinate in 50 ml Erlenmeyer flasks in the medium for varying lengths of time at 25°C. Conidia and old hyphae were also fixed directly without germination for comparison. Following the desired period of incubation, the supernatant was decanted after centrifugation, and excess liquid was drained from the tubes. The germinated conidia were then washed thoroughly in distilled water and resuspended in distilled water for at least four hours.

After the water pretreatment the material was recentrifuged and fixed in acetic acid-alcohol (1:3) for 25 minutes. Following fixation the conidia were hydrolyzed in 1 N HCl for eight minutes at 60°C. The cytoplasm of nonhydrolyzed conidia and hyphae stained so densely with orcein that nuclear details could not be critically examined.

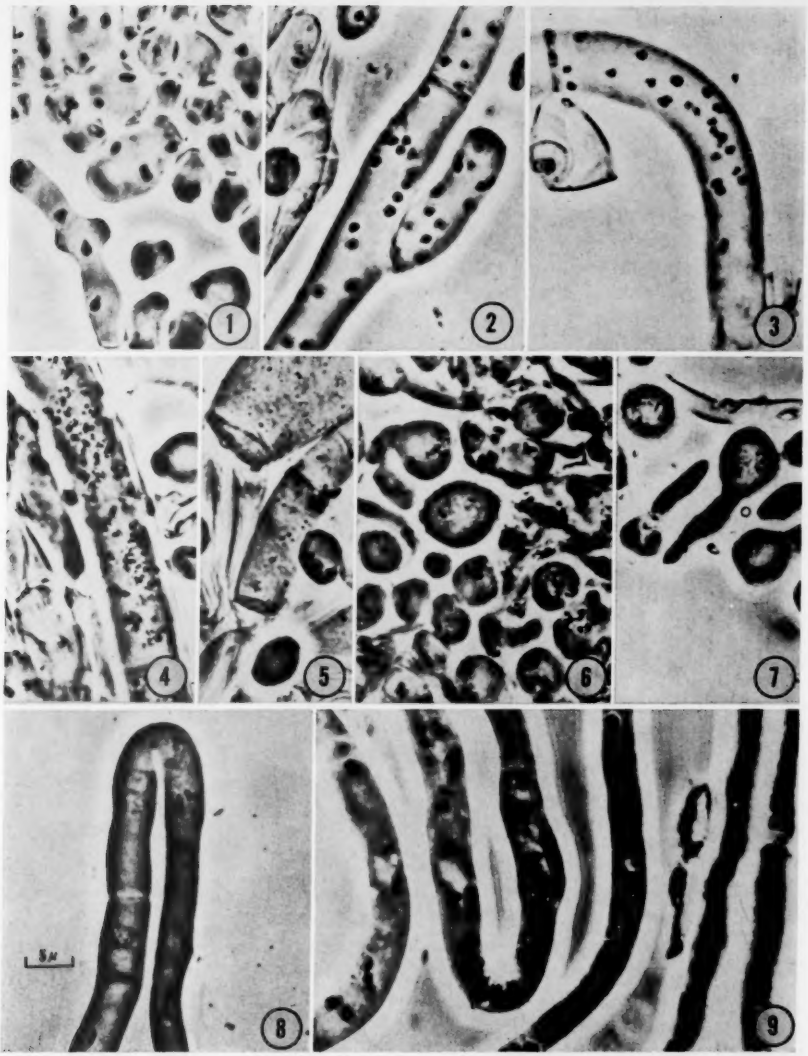
The hydrolyzed material was stained by adding a few drops of stain directly to the material in the centrifuge tubes after HCl was removed. Two percent orcein (Geo. T. Gurr, Ltd., Lot No. 11031) in 50 percent acetic acid was regularly used. However, Feulgen technique was also applied on several occasions for comparison. Squash preparations were made immediately for cytological observations. Storage of such material was performed by adopting the air-dried method suggested by ROTHFELS and SIMINOVITCH (1958) for mammalian cells. These dried preparations could be squashed with fresh aceto-orcein whenever needed.

All observations were made with either a Reichert Zetopan microscope with fixed dark-phase optics or a Reichert Biozet microscope with Variphase optics. For photographic recordings a Zeiss GF-595 microscope was routinely used. This microscope was equipped with Neofluar dark-phase objectives and Leitz Periplan oculars. All photographs were taken with a Leitz Aristophot Model 2 camera and 4″ × 5″ Ansco Hypan films. This film is a high speed negative which allows brief exposure time yet preserves good contrast comparable with some films with slower speed, such as Ansco Isopan. All films were developed in Kodak DK-60A developer at 25°C.

OBSERVATIONS

Vegetative nuclei

If mature conidia are harvested and examined directly by the aceto-orcein method just described without having been incubated for a period of time in a suitable growth medium, a variable number of from one to eight nuclei can be seen within each conidium. These dehydrated, "resting" nuclei appear as very small, densely stained bodies approximately 1μ in diameter, which in this extremely condensed state lack all structural details usually visible in interphase nuclei (Figure 1). Nuclei with a similar morphology can be observed in aerial hyphae removed from a mycelial mat which has been allowed to grow for several days in a test tube (Figures 2 and 3). If hyphae are examined from a culture



FIGURES 1–9.—Conidia and mycelia of *Neurospora crassa*, strain Em5256. Fixed with acetic alcohol, hydrolyzed in HCl, aceto-orcein squash. Dark-phase microscopy. Figures 6–9, material treated with distilled water before fixation. For magnification see scale on Figure 8. Figure 1.—Dried conidial spores directly taken from old cultures. Note the compactness of the truly "resting" nuclei, their small size and lack of structural details. Figures 2 and 3.—Resting nuclei in dried mycelia with similar characteristics as those of dried spores shown in Figure 1. Figure 4.—A dried mycelium containing degenerating nuclear material. Figure 5.—A dried mycelium containing disintegrating nuclear material shown as tiny dark spots. Figure 6.—Conidial spores immersed in liquid medium for ten hours. Note various stages of mitotic division. Compare the nuclei in this picture with those in Figures 1–3. Figure 7.—Germinating spores showing hyphal tubes and active nuclei. Figure 8.—A section of a mycelium 24 hours in culture, showing the structure of interphase nuclei. Note the heterochromatic regions in some of the nuclei. Figure 9.—Mycelia 24 hours in culture. Note interphase nuclei and some mitotic figures.

which is several weeks old, evidence of nuclear degeneration and disintegration is noted (Figures 4 and 5). Nuclear remains and fragments are of extremely variable size and shape. Since many of these fragments are within the size limits of chromosomes, it is desirable to avoid examining cultures of this age for evidence of mitosis.

If, however, dehydrated conidial spores are allowed to germinate for several hours in a suitable growth medium, cellular constituents become hydrated and active germination and growth resume. This activity is reflected by a multiplicity of mitotic figures as well as interphase nuclei (Figures 6 and 7). Furthermore, this activity can still be observed after 24 hours of incubation, for there is still an abundance of mitotic figures while interphase nuclei show their characteristic structural details (Figures 8 and 9).

Nuclear division

The various stages of nuclear division recorded from these actively growing cultures are represented by Figures 10–26. In Figures 10 and 11 characteristic round or oval interphase nuclei can be seen to vary from 2 to 3μ in diameter. Each nucleus contains a nucleolus and a prominent heterochromatic segment. While there are apparently several small regions of heterochromatin in each nucleus, the major portion is probably contributed by two large heterochromatic segments in chromosome one, the largest chromosome (SINGLETON 1953). These two segments can frequently be observed as a V-shaped figure in interphase nuclei.

Apparently prophase proceeds in the conventional manner (Figures 12–17). The chromosomes in the earliest detectable stages of prophase are more difficult to observe because of their weak stainability (Figure 12). However, while the chromosomes are as yet not highly contracted, the heterochromatin is differentially discernible. As spiralization proceeds during prophase, individual chromosomes can be observed as well as coils within chromosomes (Figures 13 and 14). Later stages of prophase showing increasing contraction of individual chromosomes are represented by Figures 15, 16, and 17.

Chromosomes, when fully contracted, assume configurations similar to regular mitotic metaphase (Figures 18 and 19). When observed from side view, individual chromosomes can be seen to align on a metaphase plate, and from a polar view of this stage morphology of individual chromosomes may be distinguished.

Very early anaphasic figures in which all chromosomes could be counted have not been recognized. However, fairly early anaphase figures have been recorded in which the ends of the longest chromosomes extend from two groups of separating daughter chromosomes (Figure 20). In Figure 21 two anaphases are evident. Observations made on late anaphasic and early telophasic figures suggest the presence of a spindle (Figures 22 and 24).

Formation of nucleoli is readily recognized during telophase, and frequently the nucleolus can be detected at one side of each chromosome group (Figure 23). One aspect of telophase in Neurospora, which seems to differ from similar stages in other organisms, is that during the stage of despiralization of chromosomes

FIGURES 10–26.—Mitotic stages in *Neurospora crassa*, strain Em5256. Treated with distilled water, fixed with acetic alcohol, hydrolyzed in HCl, aceto-orcein squash. Dark-phase microscopy. For magnification for all pictures except Figure 22, see scale on Figure 10. Magnification scale on Figure 22 for this picture only. Figures 10 and 11.—Interphase nuclei in mycelia. Note heterochromatin and nucleoli. Figure 12.—An early prophase nucleus showing a conspicuous heterochromatic segment. Figures 13 and 14.—Prophases. Figure 15.— Late prophase. Figures 16 and 17.—Prometaphases. Figures 18 and 19.—Metaphases. Figure 20.—Early anaphase. Figure 21.—A section of mycelium showing two anaphasic figures. Figure 22.—Late anaphase. Note the image of spindle indicated by the space with low contrast. Figure 23.—Telophase showing the beginning of formation of nucleoli (arrows). Figure 24.—Early telophase. Note the two chromosome groups and the image of spindle indicated by the space with low contrast. Figure 25.—Late telophase, showing the formation of elongated daughter nuclei and oval nucleoli (arrows). Figure 26.—Very late telophase, entering interphase. The nucleolus in each nucleus is now located at the center.

the daughter groups assume a much elongated, instead of the regular roundish, shape. Figure 25 is an excellent example showing the shape of the nuclei, the nucleoli and the chromosomal segments at which the nucleoli attach. The young nucleus seems to contract later and becomes more or less spherical. By this stage the nucleolus is still located acentrically, and the chromatin mass is often displaced to the other side. A pair of daughter nuclei representing very late telophase is shown in Figure 26. The nuclei are entering interphase, and at this stage the prominent nucleoli are centrally located.

Chromosome number and morphology

In the majority of metaphase figures observed, seven distinct chromosomes could be counted (Figures 27–31). However, in approximately one fourth of the figures a tiny eighth chromosome is noted (Figure 32).

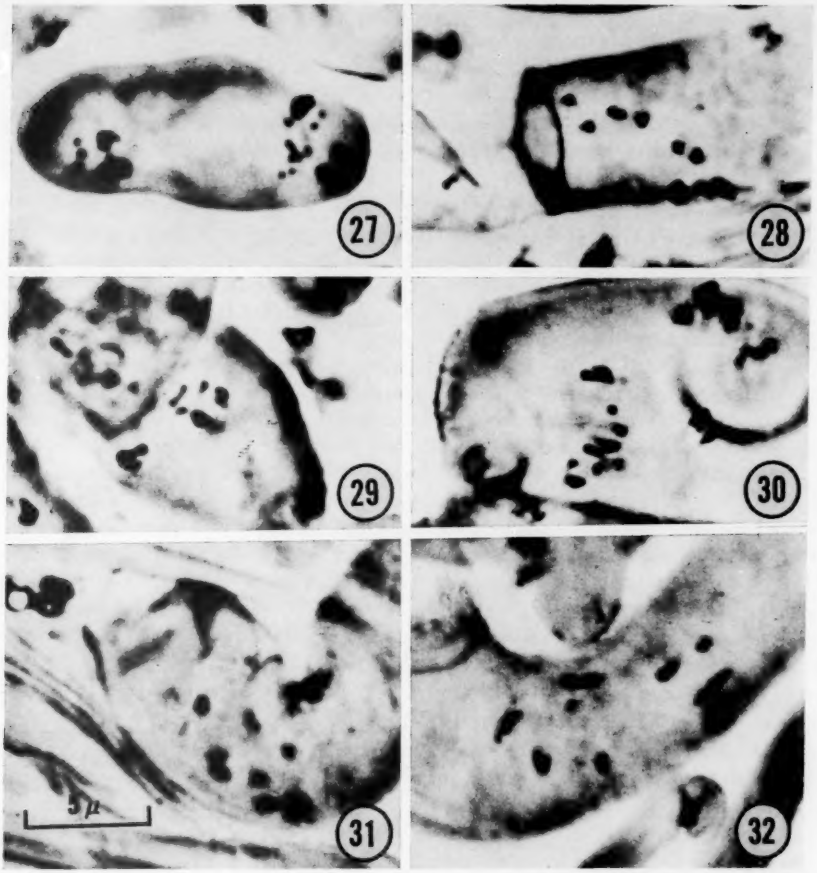
The largest chromosome, chromosome 1, is approximately 1μ in length and is characterized by having a subterminal secondary constriction. Chromosome 2 is slightly shorter in length and possesses a relatively long secondary constriction and a satellite (Figures 27 and 30). The other five major chromosomes vary slightly in length but are all noticeably shorter than chromosomes 1 and 2. These smaller chromosomes are each characterized by a single, more or less median, primary constriction. The smallest, the eighth chromosome, is approximately one tenth the size of the large chromosome 1.

DISCUSSION

The observations reported herein present evidence for division of vegetative nuclei in *N. crassa* which is similar to mitosis of somatic nuclei of other organisms. These observations are not in agreement with BAKERSPIGEL's (1959) report of a seemingly amitotic system in division of somatic nuclei of this fungus. However, it is believed that this difference lies only in interpretation, for the division figures recorded in BAKERSPIGEL's photographs are similar to those presented in this report.

For instance, in the case of interphase nuclei, the "granule" seen by BAKERSPIGEL (Figure 48, 1959) is reported here as a heterochromatic segment. Furthermore, BAKERSPIGEL's Figure 43 appears similar to prophase figures, while his Figure 13 could be interpreted as prometaphase. Similarly, his Figures 12, 14, 52, and 53, which he considers as complexes of chromosomal filaments, are somewhat comparable to metaphase configurations in which chromosomes have not been spread sufficiently widely to show their individual morphology.

In comparing our own preparations made with the Feulgen techniques observed with light microscopy and the aceto-orcein squash viewed with phase microscopy, there was no question that the latter method gave more contrast and revealed more detail. The water pretreatment, the mass culture, and the relatively heavy squashing also contributed to the preliminary success in the study of somatic nuclear division of Neurospora. Nevertheless, critical analysis of the morphology of mitotic chromosomes still awaits further improvement of



FIGURES 27–32.—Chromosomes of *Neurospora crassa*, strain Em5256. Treated with distilled water, fixed with acetic alcohol, hydrolyzed in HCl, aceto-orcein squash. Dark-phase microscopy. For magnification see scale on Figure 31. In Figure 27 a germinating conidial spore is shown with two metaphase figures. The two larger chromosomes on the right side are chromosomes 1 and 2. Chromosome 1 possesses a prominent subterminal constriction, and chromosome 2 has a satellite. Other chromosomes are two-armed. In Figure 30 chromosome 1 is located at the top of the metaphase plate. Note the constriction. Chromosome 2 is at the bottom of the plate. Note the long, secondary constriction which connects the main chromosome body and a satellite. In Figure 32, eight chromosomes can be clearly seen, one being very small.

techniques. Although the spreading of chromosomes is greatly enhanced by the distilled water pretreatment, the cell wall actually limits the dimensional expansion of the protoplasm. Perhaps this difficulty will be resolved by studying chromosomes in protoplasts obtained by the method as outlined by BACHMANN

and BONNER (1959). An attempt is being made to determine whether this could be a possible method for eliminating our present handicaps.

While other fungi have not been observed, it is not unreasonable to suggest that an improved method can be applied to assist in developing the field of cytology of fungi, which for a number of years has had conflicting reports in the literature regarding chromosome numbers and mitosis (MARTENS 1946; CUTTER 1951; OLIVE 1953).

Since it is now feasible to observe chromosome behavior and morphology in vegetative hyphae of Neurospora and to handle relatively large amounts of material for observations, cytological analysis can be made of certain aberrant genetic results obtained by several investigators. MITCHELL, PITTENGER and MITCHELL (1952) and PITTENGER (1954) postulated that the behavior of pseudo-wild types in outcrosses of *Neurospora crassa* was possibly due to an N + 1 chromosomal constitution. More recently, MITCHELL (1958, 1959) has suggested that the aberrant behavior in certain genetic recombinations may be the result of hyperhaploidy with each additional mutant chromosome being a step toward prototrophy. These hypotheses should be testable by cytological observations.

Although the first article on chromosome number of *N. crassa* reported it to vary from six to nine (LINDEGREN and RUMANN 1938), for many years seven has been accepted as the haploid set. This was mainly based upon the observations of MCCLINTOCK (1945) and SINGLETON (1953) on chromosomes in the ascus. Seven is also the number of known linkage groups in this organism (BARRATT, NEWMEYER, PERKINS and GARNJOBST 1954). However, from observations on somatic chromosomes reported here it appears that the certainty of this number is no longer firmly established. While seven also appeared in most of the cells observed in the present study, a very small eighth chromosome has been noted in several metaphase figures (Figure 32).

This eighth chromosome may be a supernumerary that occurs only in a small fraction of the nuclei of the stock employed as in the case described by MCCLINTOCK (1954), or a supernumerary chromosome particular to this strain, or even a regular member of the haploid set of *N. crassa*. With the present technique, it is rather difficult to obtain conclusive evidence because our knowledge about the morphology of the seven larger chromosomes is still far from adequate. Since several larger chromosomes possess secondary constrictions, the smallest chromosome, if it is present regularly, may easily be regarded as a part of one of the larger elements. Another possibility is that the eighth chromosome may be lying above or beneath one of the larger chromosomes so that it is not always visible. It is also possible that the eighth chromosome is largely heterochromatic in nature in addition to its small size (approximately 0.1μ in length). This would probably explain the difficulty in accounting for an eighth linkage group. However, without further improvements in cytological techniques, together with re-examination of pachytene chromosomes in the ascus, the true nature of the eighth chromosome cannot be ascertained.

SUMMARY

1. A method is outlined for the study of mitosis in *Neurospora crassa*, and observations on mitosis in a wild type strain are described.

2. The morphology and number of the metaphase chromosomes are described and discussed. While seven chromosomes are easily distinguished, it is possible that an eighth chromosome also exists.

3. The feasibility of using the method presented herein to study mutant strains of *N. crassa* which show aberrant genetic behavior is discussed.

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